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Fracture Healing in Human Immunodeficiency Virus Positive Patients

The HIV in Orthopaedic Skeletal Trauma (HOST) Study

Thesis submitted in accordance with the requirements of the University of Edinburgh for the degree of

Doctor of Philosophy

By

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Declaration

I declare that this thesis was composed solely by myself, and that it has not been submitted, in whole or in part, in any previous application for a degree. Except where states otherwise by reference or acknowledgment, the work presented is entirely my own.

Parts of this work have been published in the South African Orthopaedic Journal and International Orthopaedics and I confirm that any work included from these publications in this thesis is my own work.

Simon M Graham

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Abstract

Background: Human immunodeficiency virus (HIV) has been shown to reduce bone mineral density (BMD), mineralisation and bone turnover. In the HIV-negative population, reduced BMD is associated with delayed bone union and this may also be true in HIV infection. Previous clinical and basic science research has suggested an association between HIV infection and impaired fracture healing. However, the effect of HIV on bone healing is very poorly understood. The aim of this study was to establish whether HIV is a risk factor for the development of delayed bone union or non-union following a fracture.

Methodology: The project aims were addressed with two related clinical studies undertaken at two tertiary referral hospitals in Cape Town, South Africa.

1. **HIV in Orthopaedic Skeletal Trauma (HOST) 1 Study:** Case-cohort study of participants undergoing fracture surgery: All adult participants with fresh tibia and femur fractures who underwent IM nailing for fracture fixation were eligible for inclusion over a 14-months period. Participants were evaluated at six weeks, and three, six, nine and 12 months post-operatively. The primary outcome was delayed bone union at six months (Radiological Union Score for the Tibia [RUST] score < 9) and the secondary outcome was non-union at months 9 (RUST score < 9).

2. **HIV in Orthopaedic Skeletal Trauma (HOST) 2 Study:** Matched case-control study of participants presenting with non-unions (RUST < 9) of fractures: Adult participants (cases) with established non-unions of the femur or tibia shaft were recruited over a 14-months period and matched for: a) age; b) sex; c) fracture site; and d) fracture management type, with 'control' participants who progressed to fracture union (RUST > 9) within six months of injury.

All study participants underwent HIV testing, with measurement of CD4 cell count and viral load and a history of anti-retroviral (ART) therapy if appropriate. Bone healing was assessed by two blinded independent reviewers, using the RUST scoring system. The odds of delayed and non-union by HIV group were estimated and compared using univariate and multivariable logistic regression.

Results:

1. **HOST 1 study**: A final study population of 358/400 (89.5%) participants, who underwent 395/442 (89.4%) IM nailings were recruited over a 14-month period. All participants were followed up for a minimum of 12 months. 71 participants (71/358, 19.8%) were HIV-positive (83 IM nailings [83/395], 21.0%). HIV was not statistically significantly associated with the development of delayed bone healing following an IM nailing of the tibia or femur in this study population (univariate OR 0.76, [CI 0.37-1.44], p-value=0.417, multivariable OR 1.06 [CI 0.50-2.22], p-value=0.869). However, the HIV-positive participants had a statistically significant lower risk of non-union compared to HIV-negative (univariate OR 0.16 [CI 0.01-0.78], p-value = 0.076, multivariable OR 0.17 [CI 0.01-0.92], p-value = 0.100).

2. **HOST 2 study**: A total of 57 cases were matched with 57 controls, over a 14-month period. The prevalence of HIV among cases was 7% (4/57) and was 15.8% (9/57) among controls, with an overall prevalence of 11.4% (13/114) in the study population. HIV status was not associated with the development of non-union following the management of tibia and femur fractures, on either univariate (OR 0.40 [CI 0.10-1.32], p-value = 0.151) or multivariable (OR 0.86 [CI 0.18-3.73], p-value = 0.831) logistic regression analysis.

Conclusion: The HOST 1 and 2 studies demonstrate that HIV is not associated with the development of delayed union following fracture of the tibia or femur. Additionally, HIV-positive status appears to be associated with a lower risk of

developing a non-union. Therefore, fractures sustained in HIV-positive individuals can be managed in the same way as those who are HIV-negative, with no increased risk of delayed or non-union. Future areas of research are indicated to assess the role of ART and CD4 cell count on fracture healing.

Lay abstract

Background: Human immunodeficiency virus (HIV) has been shown to cause thin and brittle bones and reduce new bone being made. In people without HIV, thin and brittle bones can cause slow bone healing. Previous laboratory and epidemiology research have suggested an association between HIV infection and impaired bone healing. The effect of HIV on bone healing is very poorly understood. The aim of this study was to establish whether HIV causes slow bone healing or bones not to heal at all, following an injury.

Methodology:

The project aims were addressed with two related studies in two hospitals in Cape Town, South Africa.

1. **HIV in Orthopaedic Skeletal Trauma (HOST) 1 Study**: All adult participants undergoing an operation to fix their broken tibia or femur bone with a type of metal nail were eligible for inclusion in the study over a 14-month period. Participants were reviewed six weeks, and three, six, nine and 12 months after their operation. An assessment of bone healing was made to see if there was a difference in the proportion broken bones that were slow to heal or did not heal at all was different according to HIV status.

2. **HIV in Orthopaedic Skeletal Trauma (HOST) 2 Study**: Adults with bones that had not healed after sustaining a broken femur or tibia bone were recruited over a 14-month period. They were compared to adults whose bones had healed to see if there was a difference in the number of participants that had HIV.

All participants underwent HIV testing, with measurement of their treatment for HIV and blood tests if appropriate.

Results:

1. **HOST 1 Study**: A final study population of 358 participants, who underwent 395 operations for a fracture, were recruited over a 14-month period. 71 participants, 19.8% of the whole study population, had HIV. On analysis, HIV was shown not to be a risk factor for the development of slow bone healing. Additionally, HIV-positive fracture patients were less likely to experience non-union.

2. **HOST 2 Study**: A total of 57 participants whose bones had not healed were compared to 57 participants whose bones had healed over a 14-month period. There was a similar number of people with HIV whose bones had healed compared to those whose had not. Therefore, suggesting HIV did not cause a problem with bone healing in the study population.

Conclusion: The HOST 1 and 2 studies demonstrate that HIV does not cause bones to heal up more slowly and may even improve bone healing. Therefore, fractures sustained in HIV-positive patients can be managed in the same way as those who are HIV-negative, with no increased risk of problems with bone healings. Future areas of research are indicated to assess the role of HIV treatment on fracture healing.

Publications

Publications related to information presented in this thesis. Contributions from all the authors is acknowledged;

- Graham SM, Harrison WJ, Lalloo DG, Simpson AH, Laubscher M, Held M, Ferreira N, Maqungo S. HOST Study – HIV in Orthopaedic Skeletal Trauma Study: protocol for a multicentre case-cohort study. South African Orthopaedic Journal. 2018;17(3) DOI 10.17159/2309-8309/2018/v17n3a7
- Wijesekera MP, Graham SM, Harrison WJ, Lalloo DG, Simpson AH. Fracture management in HIV-positive individuals: a systematic review. International Orthopaedics. 2016 Dec;40(12):2429-2445. Epub 2016 Sep 21.

Abbreviations

AIDS	Autoimmune Deficiency Syndrome
ART	Anti-retroviral therapy
BMP	Bone morphogenetic protein
BMI	Body mass index
CT	Computed tomography
CI	Confidence interval
DALY	Daily adjusted life years
DEXA	Dual energy X-ray absorptiometry
DKK	Dickkopf-related protein
DRI	Disability related index
DSI	Deep surgical site infection
FDA	Food and drug administration
FI	Fusion inhibitor
FZD4	Frizzled-4
GA	Gustilo Anderson
GDP	Gross domestic product
GP	Glycoprotein
GPS	Global Positioning System
GSH	Groote Schuur Hospital
GSW	Gunshot wound
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HOST	HIV in Orthopaedic Skeletal Trauma
ICC	Initial class correlation
IL	Interleukin
IM	Intramedullary nailing
IQR	Interquartile range
IGF-1	Insulin-like growth factor-1

IFNg	Interferon gamma
ISS	Injury severity score
LEDGF	Lens epithelium-derived growth factor
LMIC	Low- and middle-income countries
LRP	Lipoprotein receptor-related protein
ML	Maritz Laubscher
M-CSF	Macrophage colony- stimulating factor
MSC	Mesenchymal stem cell
NHLS	National Health Laboratory Service
NF	Nando Ferreira
NY	Nomsa Yekiso
NM	Nosipho Mncwabe
NSAIDs	Nonsteroidal anti-inflammatory drugs
PICO	Population, intervention, comparator and outcome
PTH	Parathyroid hormone
PrEP	Pre-Exposure Prophylaxis
PROM	Patient reported outcome measurements
OPG	Osteoprotegerin
OR	Odds ratio
ORIF	Open reduction and internal fixation
QECH	Queen Elizabeth Central Hospital
RANKL	Receptor activator of nuclear factor kappa-B ligand
RNA	Ribonucleic acid
RR	Relative risk
RSA	Republic of South Africa
RTI	Reverse transcriptase inhibitor
RUST	Radiographic Union Scale for Tibia
SOCS-1	Suppressor of cytokine signaling- 1
SSI	Surgical site infection
TBH	Tygerberg Hospital

TB	Tuberculosis
TGF-β	Transforming growth factor-beta
TNF-α	Tumour necrosis factor- α
TRAF-6	Tumor necrosis factor receptor-associated factor-6
U.S	United States
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

NNRTI Non-nucleoside Reverse transcriptase inhibitor

- DOR Doravine
- EFV Efavirenz
- ETR Etravirine
- RPV Rilpivirine

NRTI Nucleoside Reverse transcriptase inhibitor

- ABC Abacavir
- AZT Azidothymidine
- FTC Emtricitabine
- 3TC Lamivudine
- ZDV Zidovudine
- d4T Stavudine
- TDF Tenofovir disoproxil fumarate
- TAF Tenofovir Alafenamide

PI Protease inhibitor

- ATV Atazanavir
- APV Amprenavir
- DRV Darunavir
- IDV Idiniavir
- LPV Lopinavir
- NVP Nevirapine
- FPV Fosamprenavir
- RTV Ritonavir
- SQV Saquinavir

FI Fusion Inhibitors

- T-20 Enfuvirtide

CCR5 Antagonist C-C chemokine receptor type 5

- MVC Maraviroc

INI Integrase inhibitors

- DTG Dolutegravir
- RAL Raltegravir

IBA Ibalizumab

Table of Contents

CHAPTER 1. INTRODUCTION TO THESIS

1.1 Background

Worldwide, approximately 35.3 million people are Human Immunodeficiency Virus (HIV) positive, with the highest prevalence seen in sub-Saharan Africa.(1) The introduction of anti-retroviral therapy (ART) in 1997 has altered the course and nature of patients infected with HIV by increasing the duration of asymptomatic infection and, consequently, patients with HIV are attaining close to normal life spans.(2), (3) ART consists of a combination of drugs that aim to suppress the HIV virus replication and stop the progression of HIV disease. However, despite these longer life expectancies, there is little evidence to advise the surgeon and patient about the effect of long-term immunosuppression on the fracture repair process in orthopaedic surgery.(4)

HIV principally affects a patient's immunological status by reducing the host CD4 T cell count, resulting in an increase in the risk of a patient developing opportunistic infections. HIV has also been shown to affect other chemical mediators, including interleukins 1 and 6 (IL 1, IL6) and tumour necrosis factor (TNF), which have been shown to play a role in the fracture repair process.(5), (6), (7)

HIV and ART have both been shown to reduce bone mineral density (BMD), bone mineralisation and bone turnover in clinical studies.(8), (9), (10), (11) In the general population, it has been postulated that a reduced BMD is associated with a reduced speed of fracture healing. If this relationship were to hold true in the context of HIV, then they would not only be at an increased risk of fragility fracture, but also of subsequent delayed fracture healing and failure of fracture fixation.

A factor known to affect fracture healing is local blood flow to the site of the injury. It is now well established that HIV infection is associated with osteonecrosis, thought to be due to interruption in osseous blood supply. Although, no mechanism for this

interruption in blood supply has been shown.(12), (13), (14) ART has also been reported to contribute to this pathology.(12) Conditions that jeopardise arterial flow to the site of primary bone healing are associated with higher rates of delayed fracture healing and non-union.(15), (16), (17)

A small number of studies have investigated the role of HIV in the fracture healing process. These have suggested that HIV and/or ART are associated with delayed fracture healing and may result in non-union.(18), (19) The molecular and cellular mechanisms driving this process remain unclear and the effect of HIV and ART on bone healing is very poorly understood.

Our study group has previously demonstrated there may be an association between HIV infection and impaired fracture healing. (18), (20) Furthermore, analysis of patients presenting to Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi, for immediate fracture treatment and non-union surgery, indicates twice the frequency of HIV seropositivity in the non-union group (Figure 1-1). (Personal communication - Professor WJ Harrison) Other researchers have suggested that HIV may impair fracture healing, based on extrapolation from basic science and laboratory research. (19)

These observations prompted this study, as the effect of HIV on bone healing is very poorly understood and has not previously been thoroughly investigated.

1.2 Primary research question

Is HIV infection a risk factor for the development of delayed bone union or non-union following a fracture?

1.3 Aim

To establish whether HIV is a risk factor for the development of delayed bone union or non-union following a fracture.

The project aims were addressed with two related clinical studies undertaken at two tertiary referral hospitals in Cape Town, South Africa

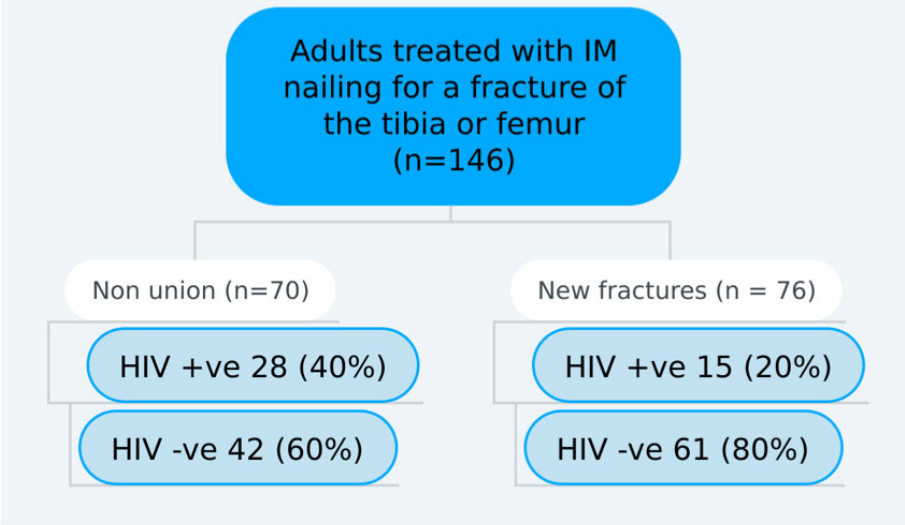
1. **HIV in Orthopaedic Skeletal Trauma (HOST) 1 Study:** Case-cohort study of participants undergoing intramedullary nailing of the tibia and femur for fracture fixation.
2. **HIV in Orthopaedic Skeletal Trauma (HOST) 2 Study:** Case-control study to investigate risk factors for non-union of fractures of the femur or tibia

1.4 Hypothesis

HIV is a risk factor for the development of delayed bone union and non-union following a fracture.

If this hypothesis were shown to be true, the surgical management of fractures could be tailored to optimise bone union during the fracture-healing phase in HIV-positive patients, improving outcomes and reducing the substantial physical and social burden that occurs in these patients as a result of traumatic injuries.(18)

Figure 1-1. The HIV status of patients presenting to Queen Elizabeth Central Hospital, Blantyre Malawi for intramedullary nailing (IM) with new fractures and non-unions: 2005 – 2007



1.5 Background to South Africa, demographics, socioeconomics and health care

1.5.1 South Africa

South Africa, officially named the Republic of South Africa (RSA), is located at the southernmost region in Africa. It is bordered to the south by 2,798 kilometres of coastline of Southern Africa, extending along the South Atlantic and Indian Oceans (Figure 1-2).(21) To the north, it borders Namibia, Botswana and Zimbabwe, and to the east of the country, Mozambique and Swaziland. Additionally, it also surrounds the country of Lesotho.(21)

Figure 1-2. Map of South Africa. (22)

1.5.2 Demographics

South Africa has an estimated population of 56,015,000 (2016), is the largest country in Southern Africa and the 25th largest in the world.(21) There are also an estimated five million living illegally in South Africa, the majority of which are from Zimbabwe.(21) The 2011 census defined five main racial groups living in South African. These include Black African at 79.2%; White at 8.9%; Coloured at 8.9%; Asian at 2.5%; with other racial groups making up the final 0.5%.(21) There are 11 official languages in South Africa. These include: Afrikaans; English, Zulu; Xhosa;; Northern Sotho; Tswana; Tsonga; Southern Sotho; Swazi; Venda and Southern Ndebele.(21) The three most commonly spoken first languages are Zulu (22.7%), Xhosa (16.0%), and Afrikaans (13.5%).(21) This creates an extremely diverse population group of people living in the South Africa and is the reason why the country is referred to as the 'Rainbow Nation'.(21)

1.5.3 Socioeconomics

According to the World Bank, South Africa is an upper middle-income emerging economy and the second largest economy in Africa, after Nigeria.(23) The country has an abundant supply of natural resources, well-developed financial, legal, communications, energy and transport sectors, and a stock exchange that is Africa's largest (and among the top 20 in the world). It also has a relatively high gross domestic product (GDP) per capita compared to other countries in sub-Saharan Africa. Despite this, South Africa is still burdened by a relatively high rate of poverty and unemployment and is also ranked in the top ten countries in the world for income inequality.(23)

Unlike many of the world's low- and middle-income countries, South Africa does not have a thriving informal economy, with only 15% of South African jobs in the informal sector of work. This is very different to a similar upper middle-income country, such

as Brazil, where approximately 50% of the jobs are in the informal sector. Official unemployment is roughly 27% of the workforce.(3)

More than ten million people live on less than $1 per day,(3) which is the so-called food poverty line, below which people are unable to purchase enough food for an adequate diet. Additionally, a further 45% of the population in South Africa live on approximately $2 per day (the upper limit for the definition of poverty).(3) This is in contrast to the top 10% of South Africans who earn 58% of the total annual national income, whereas the bottom 70% combined earn a only 17%,(3) creating one of the widest economic disparities in the world and one of the unique characteristics of South Africa's current socio-economic climate.

1.5.4 Health care

The health system comprises the public government sector and the private sector. The public health services are divided into primary, secondary and tertiary through health facilities that are located in, and managed by, the provincial departments of health. The provincial departments are thus the direct employers of the health workforce, while the National Ministry of Health is responsible for policy development and coordination.(24)

South Africa's Constitution guarantees every citizen access to health services (section 27 of the Bill of Rights).(24) However, everyone can access both public and private health services, with access to private health services depending on an individual's ability to pay.(24) The private health sector provides health services through individual practitioners, who run private surgeries, or through private hospitals, which tend to be located in urban areas. The health care system consumed about 8.8% of the country's gross domestic product during 2012.(25)

The private sector serves 16% of the population while the public sector serves 84%, reflecting the void between rich and poor seen in the country.(26) The country's population distribution indicates that about 64.7% inhabit the provinces that are largely rural in nature.(26) Some of these provinces contain large cities, though the bulk of the population lives in rural communities.

The national annual per capita expenditure on health ranges from $1,400 in the private sector to approximately $140 in the public sector, and disparities in the provision of health care continue to widen every year.(3) The national public health sector, staffed by some 30% of the doctors in the country, remains the sole provider of health care for more than 40 million people who are uninsured and who constitute approximately 84% of the national population. Approximately 16% of South Africans (eight million people) have private health insurance that provides access to health care from the remaining 70% of doctors who work full-time in the private sector. Up to 25% of uninsured people pay out of pocket for private sector care. In recent years, permission for senior full-time staff in the public sector to spend a limited proportion of their time working in the private sector has diluted their public-service activities.(3)

The management of trauma and injuries in South Africa, such as tibia and femur fractures, are predominantly managed by orthopaedic surgeons. However, this service is significantly limited in the public sector and in many rural areas.(27) There are currently 0.36 orthopaedic surgeons per 100,000 uninsured population compared with 8.3 orthopaedic surgeons per insured population.(27) As stated earlier, private hospitals only cater to 16% of the population; however, between 80 and 95% of orthopaedic surgeons in the country work in the private sector at any given time.(27)

CHAPTER 2. HUMAN IMMUNODEFICENCY VIRUS AND ITS TREATMENT

2.1 Aim of this chapter

In this chapter the pathogenesis of HIV, virus staging, a summary of antiretroviral therapy (ART) treatment and the current picture of how HIV has affected South Africa will be discussed.

2.2 Overview

In order to have an understanding of the effect of HIV on fracture healing, it is essential to have a full appreciation of the pathogenesis of the virus and how it affects the human body. Additionally, having a good understanding of the different forms of ART therapy is vital, since they may not all impact fracture healing and bone metabolism to the same degree.

2.3 Human Immunodeficiency Virus

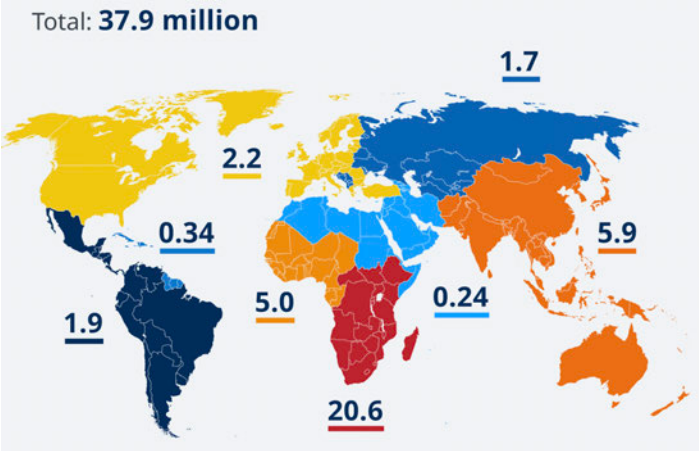
It has been shown that the HIV epidemic started after zoonotic infection with simian immunodeficiency viruses from African primates. It is likely that bushmeat hunters were probably the first group to be infected with HIV.(28) There are two main different types of HIV virus, HIV-1 and HIV-2. HIV-1 was transmitted from apes and HIV-2 from sooty mangabey monkeys.(28) Four groups of HIV-1 exist and represent three separate transmission events from chimpanzees (M, N, and O) and one from gorillas (P). Groups N, O, and P are mainly restricted to the western regional of Africa. The global HIV pandemic was caused by Group M, which started about 100 years ago and consists of nine subtypes: A–D, F–H, J and K. Subtype C predominates in Africa and India, and accounted for 48% of cases of HIV-1 in 2007 worldwide.(29) Whereas in western Europe, Americas and Australia subtype B predominates.(29) Additionally, over the last ten years circulating recombinant subtypes are becoming more common due to global movement.(29)

The reason for the predominant global infection being the HIV-1 subtype is due to the high mutation rate of this form of the virus. HIV-2 causes a similar illness to HIV-1, but immunodeficiency progresses more slowly and HIV-2 is less transmissible.(28) In this thesis the focus will be on the HIV-1 which makes up the majority of the infections in South Africa.

Globally an estimated 36.9 million people were living with HIV in 2017.(30) (Figure 2-1) Sub-Saharan Africa, particularly southern Africa, has the highest global burden of HIV, with 70·8% of HIV infected people on the planet living in this region.(30) Since the introduction of ART and the expanding access to this therapy, the global epidemiology of HIV infection has changed significantly. The global prevalence of HIV has increased from 31·0 million in 2002, to 35·3 million in 2012. This is due to the fact that people on ART are living longer, whereas estimated global incidence of Acquired immunodeficiency Syndrome (AIDS) has decreased sustainably from approximately 3·3 million new infections in 2002, to 2·3 million in 2012. (30)

HIV is a major contributor to the global burden of disease worldwide and in 2010, HIV was the leading cause of disability-adjusted life years for people aged 30–44 years, and the fifth leading cause for all ages.(31)

Figure 2-1. The estimated number of people living with HIV around the word in 2018 – World Health Organisation. (30)



2.4 Pathogenesis of Human Immunodeficiency Virus

HIV is a retrovirus that specifically targets CD4+ T lymphocyte cells within the immune system of an infected individual. The role of the CD4+ T cells is to formulate the immune response to infection by identifying foreign antigens and then generating an antibody response.(32), (33) HIV-1 thrives by taking advantage of cellular pathways while neutralising and hiding from the different components of the immune system so the body cannot fight the infection.(33), (34)

The lifecycle of HIV-1 is very complex (Figure 2-2) and its duration and outcome is dependent on the cell activation and target cell type.(35) In the early stage of infection, HIV-1 gains access to cells in the body without causing immediate lethal damage. However, the entry process can stimulate intracellular signal cascades, which as a result might facilitate viral replication.(36)

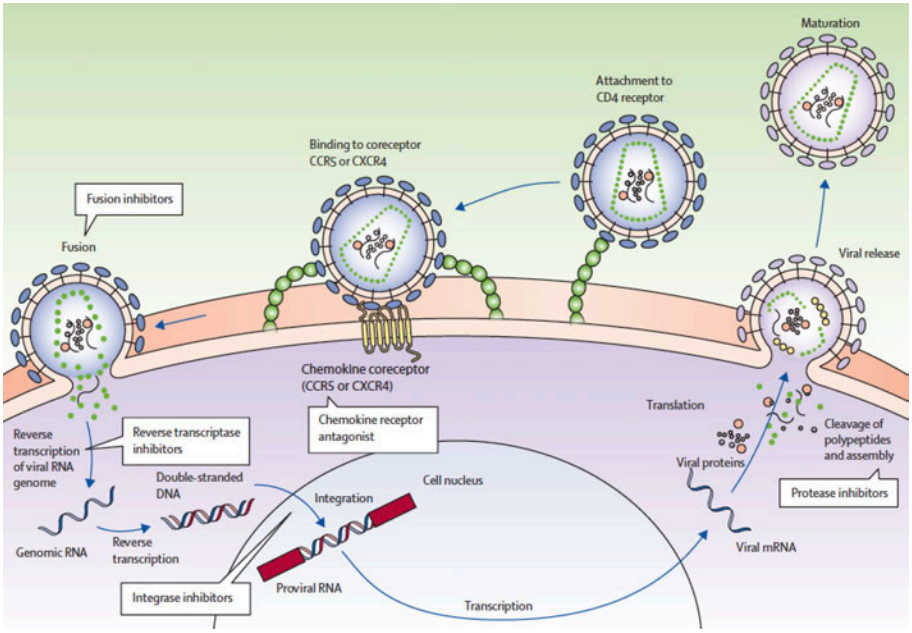
There are two molecules on the HIV-1 envelope, the external glycoprotein (gp120) and the transmembrane protein (gp41). These molecules form the spikes on the virion's surface.(34) During the virus's entry process into the CD4+ T cells, gp120

attaches to the cell membrane by first binding to the CD4+ receptor. Subsequent interactions between virus and chemokine co-receptors (e.g. CCR5, CXCR4) trigger irreversible conformational changes to the cell.(37), (38) The actual fusion event takes place within minutes by pore formation,(38), (39) and releases the viral core into the cell cytoplasm. After the core disassembles, the viral genome is reverse transcribed into DNA by the virus's own reverse transcriptase enzyme.(33)

At the middle stage of HIV infection, the viral protein integrase, in combination with host DNA repair enzymes, inserts the viral genome into gene-rich, transcriptionally active domains of the host's chromosomal DNA.(40), (41),(42) This results in irreversibly transforming the cell into a potential virus producer.

In the late stage, viral proteins are transported to, and assemble in proximity to, the cell membrane. (33) Virus egress from the cell is not lytic and takes advantage of the vesicular sorting pathway, which normally mediates the budding of endosomes into multivesicular bodies.(43) HIV-1 uses this protein-sorting pathway, finally producing mature infectious virions.(44), (45)

Figure 2-2. HIV lifecycle and sites of action of different classes of antiretroviral drugs.(34)



Since cytoplasmic molecules of the producer cell and components from its cell surface lipid bilayer are combined into the new viral particle, virions have characteristics of the cells in which they were produced. This results in any detection by the immune system difficult.(46) These virus particles are released from the infected CD4+ T cell and ultimately enter the blood or extracellular fluid and go on to infect another CD4+ T cell, spreading the infection throughout the body.(46)

In summary, HIV attaches to receptors on the CD4+ T cell, and once fused, incorporates RNA into the host cell's DNA. The newly-infected CD4+ T cell then replicates its DNA to reproduce large numbers of HIV virions within the host, which are released into the bloodstream with a cascade effect.(32) The infected CD4+ cells rapidly become ineffective and when left untreated an infected individuals immune system deteriorates to become dangerously weak. As a result, HIV-positive individuals become susceptible to severe opportunistic infections from organisms that would ordinarily be harmless with the final disease stage being AIDS.(47)

The severity of an individual's HIV infection can be measured by the 'viral load', which measures the amount of virus found within the host's bloodstream.(32) Patients with a high viral load have overwhelming HIV infection, which causes rapid CD4+ T cell destruction and a low 'CD4+ count' (<350 per microliter).(32) HIV can be separated into three phases of infection, commonly referred to as the primary infection phase, latency phase and the overt AIDS phase.(33)

The primary infection phase occurs when the infected host creates antibodies in response to an HIV infection. This normally occurs within the first 45 days of infection. During this time, individuals may have mild flu-like symptoms, headache, diarrhoea and a fever, or may have no symptoms at all, a process called seroconversion. Viral load figures are high in primary infection, sometimes greater than one million copies/ml. While CD4 count falls below normal range of 800–1000 cells/µL.(33)

Succeeding seroconversion, patients may live unaware they have HIV for 10 or more years, and ultimately have very few symptoms. However, HIV remains active within the infected host, replicating and injuring their immune system and an individual can pass on the virus spreading the infection. During HIV latency, the viral load will remain high, and CD4 cell counts fall to low levels of around 200 cells/µL or less.(33)

If HIV is left untreated, opportunistic infections and/or malignancy will develop, and those who have a CD4 count of less than 200 cells/µL with a AIDs defining illness are said to have developed AIDS.(33) This is commonly referred to as advanced or late-stage HIV. Without treatment, patients with AIDS will likely die within two years as a result of persistent and recurrent infection, such as tuberculosis (TB), pneumonia and severe fungal infections.(33)

2.5 Antiretroviral therapy

In the late 1990s, combination ART drug regimens that were able to suppress viral replication were developed and made available for the treatment of HIV. This completely revolutionised HIV from a progressive illness with definite fatal outcomes into a chronic manageable disease.(34) There are currently more than 25 licensed ART drugs available, in seven different categories. They block HIV replication at many different steps in the virus lifecycle. (Figure 2-2) The overall aim of ART is to reduce the host's viral load to undetectable levels (<40 copies per microlitre) to inhibit HIV replication and limit immune system injury to avoid the disease progression.

The introduction and widespread use of ART have significantly altered the natural history of HIV infection. However, taking ART can lead to serious medication-related adverse effects. These include an increased risk of developing cardiovascular disease, lipodystrophy, diabetes, insulin resistance and hyperlactatemia/lactic acidosis.(48) Additionally, a number of ARTs drugs have been implicated in the emerging evidence that the therapy plays a role in HIV-associated bone disease.

Current recommended combination ART regimens are less toxic, are more effective, have a lower pill burden, and are dosed less frequently than those first introduced and developed in the early 1990's. (Table 2-1)

Table 2-1. United States (U.S.) Food and Drug Administration (FDA) approved ART Drugs.(49)

Drug Class	Generic Name
Nucleotide Reverse Transcriptase Inhibitors (NRTIs)	
NRTIs present the synthesis of double-stranded DNA, acting as a chain terminator that prevents multiplication of HIV.	Abacavir (ABC) Emtricitabine (FTC) Tenofovir (TDF) Zidovudine or azidothymidine (AZT, ZDV) Lamivudine (3TC)
Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs)	
NNRTIs bind to and alter reverse transcriptase, an enzyme HIV needs to make copies of itself. Therefore, impeding the ability to convert HIV RNA to DNA, preventing replication.	Doravirine (DOR) Efavirenz (EFV) Etravirine (ETR) Nevirapine (NVP) Rilpvirine (RPV)
Protease Inhibitors (PIs)	
PIs block HIV protease, an enzyme HIV needs to make copies of itself.	Atazanavir (ATV) Darunavir (DRV) Fosamprenavir (FPV) Ritonavir (RTV) Saquninavir (SQV) Tipranivir (TPV)
Fusion Inhibitors (FIs)	
FIs block HIV from entering the CD4 cells of the immune system. Meaning it cannot infect the cell.	Enfuvirtide (T-20)
CCR5 Antagonists	
CCR5 Antagonists block CCR5 coreceptors on the surface of certain immune cells that HIV needs to enter cells.	Maraviroc (MVC)

Integrase inhibitors (IIs)	
IIs block HIV integrase, an enzyme HIV needs to make copies of itself.	Dolutegravir (DTG) Raltegravir (RAL)
Ibalizumab (IBA)	
Post-attachment inhibitors block CD4 receptors on the surface of certain immune cells that HIV needs to enter the cells.	Ibalizumab (IBA)

The WHO recommends that all adults, adolescents and children with HIV at any CD4 cell count or disease stage commence on ART drugs. They recommend a combination of two NRTIs, with the NNTRI (TDF + 3TC (or FTC) + EFV), although these recommendations are being continuously updated on a yearly basis with ever increasing research.(50) This is the approach and regimen that is currently used in South Africa for the management of patients with HIV at the time of the study. There are a number of varying combinations that are used as second line therapy and also for children, adolescents and pregnant individuals. After initiation of ART, the plasma viral load decreases to concentrations below the lower limit of detection, usually within three months.(34) In contrast, the recovery of CD4 T cells in individuals on ART is variable and can take up to six months.(51)

2.6 HIV in South Africa

South Africa, with 0.7% of the world's population, accounts for 17% of the global burden of HIV infection.(52) Currently, there an estimated 7.2 million people living with HIV and South Africa accounts for a third of all new HIV infections in southern Africa.(53) In 2016, there were 270,000 new HIV infections and 110,000 AIDS-related deaths. However, since 2010, new HIV infections have decreased by 49% and AIDS-related deaths have decreased by 29%.(53)

South Africa has the largest HIV treatment programme in the world, accounting for 20% of people on ART therapy globally. In 2018, UNAIDS reported that 4.4 million people were receiving treatment in South Africa. This equates to 61% of the people living with HIV in the country.(53) The country also has one of the largest domestically funded programmes in the world, with about 80% of the AIDS response funded by the government.

In 2003, the government introduced this treatment programme to provide ART to all patients with HIV infection.(54) Spending on HIV increased at an average annual rate of 48.2% between 1999 and 2005.(54) The level of growth was consistently higher than that in other areas of national health expenditure and has continued at an annual rate of approximately 25%, with dedicated HIV funding estimated at $400 million (in U.S. dollars) per annum.(54)

The success of this ART programme is evident in the increases in national life expectancy in South Africa, rising from 61.2 years in 2010 to 67.7 years in 2015.(55) Today, HIV-positive individuals in South Africa have a near-normal life expectancy, provided they are started on ART before their CD4 count drops below 200 cells per cubic millimetre.(3)

Despite the steps made to treat HIV, its prevalence remains high (18.9%) among the South African general population, although it varies markedly between regions.(56) For example, despite a national prevalence of 18.9%, there is a rate of 6.8 and 5.6% in Northern Cape and Western Cape, respectively.(57), (58)

As mentioned earlier, throughout South Africa, including the Western Cape, the WHO guidelines for the treatment of HIV are followed, using TDF + 3TC (or FTC) + EFV) as first line therapy.(50)

CHAPTER 3. FRACTURE HEALING

3.1 Aims of this chapter

In this chapter a discussion of the pathogenesis of fracture healing, epidemiology and factors that influence bone healing will be presented.

3.2 Overview

Fracture healing is a multifactorial process affected by a number of biological factors, injury characteristics, management strategies and the mechanics of the fracture fixation.(59) The multifaceted system of bone healing can be simplified into several stages of healing, beginning with hematoma formation, followed by inflammatory response, cell proliferation and differentiation, and finally ossification with subsequent remodeling of the new bone.

3.3 Epidemiology of fractures

Traumatic injury is a major cause of global mortality and disability. Worldwide, trauma-related deaths exceed those from HIV/AIDS, malaria and tuberculosis combined.(60) Additionally, these injuries occur at disproportionately higher rates in low-income and middle-income countries (LMIC), with 83% of the 4.6 million global deaths from injury occurring in LMICs.(60), (61), (62) For every trauma-related death, many more nonfatal injuries occur, including fractures.(63) It is estimated that for every injury-related death, 10 to 50 people sustain temporary or permanent disabilities. Furthermore, traumatic injuries result in more than 220 million disability adjusted life years (DALYs) lost each year in LMICs.(60)

The resulting disability can be especially crippling for the poorest patients, who experience a vicious cycle of poverty from health-care costs and decreased productivity.(64) Since there are an increasing number of road related accidents, the

incidence of musculoskeletal injury is increasing in LMICs.(65), (66), (67), (68) Added to this, delayed treatment worsens the burden of trauma-related disability in poor resource settings.(69), (70)

Globally, despite injury-related fractures constituting a major drain on health-care resources (71), (72), national epidemiological data for fracture incidence rates are lacking. Countries without such data, including South Africa, have to infer statistics based on results from other regions, in a high-income setting, which is highly problematic because of substantial variations in incidence rates.

A increasingly common cause of injury and death in South Africa is interpersonal violence with guns, with growing numbers of fractures as a result of gunshot wound (GSW) injuries.(73) The number of gun and firearm-related crimes, injuries and deaths continues to increase worldwide, with a reported rise in firearm offences of 23% in the United Kingdom (UK)(74) and 9% in the United States of America (USA)(75) between 2015 to 2016. In total, gun-related violence kills over an estimated 250,000 people each year and injures millions of others worldwide everyday.(76)

While the mortality rate attributable to firearms in SA is high (31.1 per 100,000 national and 41 per 100,000 in Cape Town (73), the burden of non-fatal firearm-related injuries is significantly worse. The SA government has not released disaggregated statistics on violent crime involving firearms or gun-related injuries in over a decade. Allard et al suggested that 127,000 non-fatal GSWs occurred per annum across the country in 2005.(77) In a study in Cape Town, from one of the recruiting hospitals for the patients in this study, Martin et al reported that treating an orthopaedic GSW patient costs USD 2,940 per injury, uses 194 minutes of theatre time, and the patient occupies a hospital bed for an average 9.75 days.(73) As a result, injuries as a result of GSW cost the SA public health sector over 13 times the national annual average expense per patient.(73)

With increasing burden of trauma and interpersonal violence and the fact that the highest rate of injury occurs in the working age population, this results in a substantial loss of earnings for households, societies and countries as a whole. An estimated US$180 billion is likely to be lost annually due to injury in LMICs.(60) This is occurring in populations with an expanding number of people living longer with HIV and having a higher risk of injury during their lifetime. Therefore, a better understanding of fracture healing in patients with HIV is important.

3.4 The biology of fracture healing

The biology of fracture healing is a complex biological process. Four main bone components contribute to the healing process: the injury site, including the cortex, the periosteum, the bone marrow and the external soft tissues. Following the initial trauma, bone heals by either direct/primary osteoneal or indirect/secondary fracture healing, which consists of both intramembranous and endochondral bone formation. Secondary bone healing is the most common pathway, since primary bone healing requires an anatomical reduction and rigidly stable conditions, commonly only obtained by open reduction and internal fixation. In all other non-stable conditions, bone heals via secondary healing.

3.4.1 Primary fracture healing

Primary bone healing, also referred to as direct bone healing, only occurs when there has been anatomical reduction and interfragmentary compression of a fracture, leading to no motion between the fracture surfaces under functional load (absolute stability). When the correct requirements are achieved, primary bone healing can occur by direct remodelling of lamellar bone, the Haversian canals and blood vessels.(78) Primary healing of fractures can either occur through contact healing or gap healing. Both processes involve an attempt to re-establish an anatomically correct and biomechanically competent lamellar bone structure directly.(78)

3.4.1.1 Contact healing

When a fracture gap between bone ends is less than 0.01 mm and interfragmentary strain is less than 2%, a fracture heals by contact healing.(79) Under these conditions, cutting cones are formed at the ends of the osteons closest to the fracture site.(78) The tips of the cutting cones consist of osteoclasts, which cross the fracture line. These cavities are then filled by bone produced by osteoblasts residing at the end of the cutting cone. Consequently, this results in the simultaneous generation of a bony union and the restoration of Haversian systems formed in an axial direction.(78) The bridging osteons later mature by direct remodelling into lamellar bone resulting in fracture healing without the formation of periosteal callus.

3.4.1.2 Gap healing

Gap healing differs from contact healing in that bony union and Haversian remodelling do not occur at the same time. It occurs if stable conditions and an anatomical reduction are achieved, but the gap between the bones is less than 800 µm.(78) In this process the fracture site is primarily filled by lamellar bone oriented perpendicular to the long axis, requiring a secondary osteonal reconstruction, unlike the process of contact healing. The primary bone structure is then gradually replaced by longitudinal revascularised osteons carrying osteoprogenitor cells, which differentiate into osteoblasts and produce lamellar bone on each surface of the gap.(78) This lamellar bone, however, is laid down perpendicular to the long axis and is mechanically weak. This initial process takes approximately three and eight weeks, after which a secondary remodelling resembling the contact healing cascade with cutting cones takes place.

3.4.2 Secondary fracture healing

Secondary fracture healing, also referred to as indirect hone healing, consists of callus formation by both endochondral and intramembranous bone healing with subsequent callus formation.(80) It commonly occurs in non-operative fracture treatment and in certain operative fracture fixation methods in which some motion occurs at the fracture site, including intramedullary nailing, external fixation, or 'biological' internal fixation of complicated comminuted fracture.(81), (82)

3.4.2.1 Intramembranous ossification

Intramembranous ossification involves the formation of bone directly, without first forming cartilage, from committed osteoprogenitor and undifferentiated mesenchymal cells that reside in the periosteum, farther from the fracture site.(83) It results in callus formation, described histologically as 'hard callus'.(83) In this type of healing, the bone marrow's contribution to the formation of bone is during the early phase of healing, when endothelial cells transform into polymorphic cells, which subsequently express an osteoblastic phenotype.(83)

3.4.2.2 Endochondral ossification

Endochondral ossification involves the recruitment, proliferation and differentiation of undifferentiated mesenchymal cells into cartilage, which becomes calcified and eventually replaced by bone. Its chronological characteristics include a number of identifiable stages, including an initial stage of haematoma formation and inflammation, subsequent angiogenesis and formation of cartilage, cartilage calcification, cartilage removal, bone formation, and, ultimately, bone remodelling.
This type of fracture healing is contributed from the adjacent to the fracture periosteum and the external soft tissues, such as the muscle, providing an early bridging callus, histologically characterised as 'soft callus', that stabilises the fracture fragments.[23]

3.4.2.2.1 Haematoma and inflammation

Following an injury, a haematoma is immediately generated, which consists of cells from both peripheral and intramedullary blood, as well as bone marrow cells. The response causes the haematoma to coagulate in between and around the fracture ends, and within the medulla, forming a template for callus formation.(80) The injury initiates an inflammatory response, which is necessary for the healing to progress. There is an expression of proinflammatory cytokines, signalling molecules and growth factors critical for tissue regeneration. The acute inflammatory response peaks within the first 24h and is complete after seven days.

The initial proinflammatory response involves secretion of tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-11 and IL-18.(80) These factors recruit inflammatory cells and promote angiogenesis.(78) The TNF-α concentration has been shown to peak at 24h and to return to baseline within 72h post trauma.(80) During this timeframe TNF-α is expressed by macrophages and other inflammatory cells, and it is believed to mediate an effect by inducing secondary inflammatory signals, and act as a chemotactic agent to recruit necessary cells. TNF-α has also been shown *in vitro* to induce osteogenic differentiation of mesenchymal stem cells (MSCs).(84),(85)

IL-1 and IL-6 are believed to be most important ILs for fracture healing. (85), (86), (87) IL-1 expression overlaps with that of TNF-α with a biphasic mode. It is produced by macrophages in the acute phase of inflammation and induces production of IL-6 in osteoblasts, promotes the production of the primary cartilaginous callus, and also promotes angiogenesis at the injured site.(85), (86), (87) IL-6, on the other hand, is only produced during the acute phase and stimulates angiogenesis, vascular endothelial growth factor (VEGF) production, and the differentiation of osteoblasts and osteoclasts.(88)

3.4.2.2.2 Cartilaginous and periosteal bony callus

Following the formation of the primary haematoma, a fibrin-rich granulation tissue forms.(78) Within this tissue, endochondral formation occurs in between the fracture ends, and external to periosteal sites. These regions are also mechanically less stable and the cartilaginous tissue forms a soft callus, which gives the fracture a stable structure.(89) In animal models (rat, rabbit, mouse) the peak of soft callus formation occurs seven to nine days post trauma, with a peak in both type II procollagen and proteoglycan core protein extracellular markers.(83) At the same time, an intramembranous ossification response occurs subperiostally directly adjacent to the distal and proximal ends of the fracture, generating a hard callus. It is the final bridging of this central hard callus that ultimately provides the fracture with a rigid structure, which allows weight bearing.(80)

3.4.2.2.3 Mineralisation and resorption of the cartilaginous callus

In order for bone regeneration to progress, the primary soft cartilaginous callus needs to be resorbed and replaced by a hard-bony callus. As fracture callus chondrocytes proliferate, they become hypertrophic and the extracellular matrix becomes calcified. A cascade orchestrated primarily by macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG) and TNF-α initiates the resorption of this mineralised cartilage.(90) During this process, M-CSF, RANKL and OPG are also thought to help recruit bone cells and osteoclasts to form woven bone. TNF-α further promotes the recruitment MSC with osteogenic potential, but its most important role may be to initiate chondrocyte apoptosis.(90) The calcification mechanism involves the role of mitochondria, which accumulate calcium containing granules created in the hypoxic fracture environment.

After expansion into the cytoplasm of fracture callus chondrocytes, calcium granules are transported into the extracellular matrix, where they precipitate with phosphate and form initial mineral deposits. These deposits of calcium and phosphate become the nidus for homogeneous nucleation and the formation of apatite crystals.(78) The

peak of the hard callus formation is usually reached by day 14 in animal models.(83) As the hard callus formation progresses and the calcified cartilage is replaced with woven bone, the callus becomes more solid and mechanically rigid.(80)

3.4.2.2.4 Bone Remodelling

Finally, the fracture healing cascade initiates a second resorptive phase, this time to remodel the hard callus into a lamellar bone structure. This final bone remodelling phase is biochemically coordinated by IL-1 and TNF-α.(91), (92) Furthermore, some bone morphogenic proteins (BMPs), such as BMP2, have been postulated to also be involved in this phase.

The remodelling process is carried out by an equilibrium of hard callus resorption by osteoclasts, and lamellar bone deposition by osteoblasts. Although the process is initiated as early as three to four weeks in animal and human models, the remodelling may take years to be completed to achieve a fully regenerated bone structure following an injury.(93)

3.5 Non-union and delayed union

There is a lack of agreement amongst orthopaedic surgeons regarding the assessment of fracture healing and definitions of delayed union and non-union. Due to the varying definitions, this influences the decision to intervene in an effort to promote fracture healing, a factor particularly important in a resource limited setting. It also creates challenges in assessing outcomes across studies reporting fracture healing data.

A survey of 444 orthopaedic surgeons, response rate 77%, demonstrated definitions of delayed union ranging from one to eight months, whereas definitions of non-union ranged from two to twelve months.(94) There was considerable disagreement about both clinical and radiographic criteria to define fracture union as well as the average time required for diagnosis of delayed or non-union. The same results were reported by a systematic review of 123 studies. (95) Researchers concluded that there is a lack of consensus with regard to the definition of fracture-healing in the current orthopaedic literature.(95)

While no standardised definition of non-union exists among orthopaedic surgeons, the Food and Drug Administration (FDA) defines non-union as a fracture that persists for a minimum of nine months without signs of healing for at least three months.(96) This definition was not intended for clinical use, but was specifically devised for the testing and comparison of medical devices. It does, however, remain the most widely used definition of non-union in clinical practice.

Megas et al (97) defined non-union as a cessation of all reparative processes of healing without bone union, while Marsh et al (98) specifically emphasised the cessation of both the periosteal and endosteal healing responses without bony bridging. Many authors have suggested more pragmatic working definitions. Harwood et al defined non-union as symptomatic fractures with no apparent

potential to heal without intervention.(99) Jones et al (100) and Brinker et al (101) defined non-union as the point normal biological healing ceases and will not continue without surgical intervention. While Wiss et al (102) suggested that the designation of a non-union be made once the surgeon believes the fracture has little or no potential to heal. Although these definitions are not limited by temporal restrictions and are more directed toward clinical use, they are, however, dependent on surgeon experience to predict fracture healing.

The Judet and Judet classification, modified by Weber and Cech in 1976, classified non-unions according to the vascularity of the bone ends.(103) The vascularity was determined based on strontium-85 uptake at the fracture site to delineate the viability of the bone ends.(103) Bone scintigraphy examinations are not widely used to diagnose non-unions today and are especially difficult to perform in resource-limited settings. The amount of fracture callus visible on normal radiographs is therefore currently used as a surrogate marker for fracture site vascularity. (97). (104) Subsequently, non-unions are divided into two main categories of hypertrophic non-union and atrophic non-union.(105), (106)

In hypervascular non-union, also known as hypertrophic non-union, fracture ends are vascular and are capable of biological activity. There is evidence of callus formation around the fracture site on radiographs and it is thought to be in response to excessive micromotion at the fracture site.(107) Avascular non-union, also known as atrophic non-union, is caused by avascularity or poor blood supply of the fracture ends.(108), (109) There is no or little callus formation on x-ray and fracture line remains visible. This type of non-union requires biological enhancement in addition to adequate immobilisation to heal.(105) Oligotrophic non-unions are thought to have a similar blood supply to hypertrophic non-unions and have the potential to heal (110), but possess an inadequate healing response characterised by little or no callus formation commonly due to position or displacement of the fracture ends. However,

atrophic and oligotrophic non-unions often have the same radiographic appearance.(111)

Delayed bone healing is as difficult to define as non-union. Delayed healing corresponds to a fracture that shows slower progression to healing than anticipated, but does not necessary progress to non-union.(112), (113) However, this will vary depending on the site of the fracture; with reports of average union times of approximately 16 weeks for the tibia and 12 weeks for the femur being reported, although a number of ranges have been suggested.(112), (113), (114), (115) However, this depends on a number of parameters, as discussed in this chapter.

Commonly suggested ranges for an overall risk of non-union have been suggested and the commonly quoted rates of 5-10% does not arise from any clear data or previous study, despite being highlighted in many scientific papers.(116) Varying non-union rates are found in different types of fractures, ranging from up to 3-22% in the tibia diaphysis (117), (118) to 2-10% in the femoral shaft after reamed nailing.(119)

3.6 Classification of fracture healing

Determining a fracture has healed requires radiologic and clinical assessment. However, as discussed there is no uniform definition of fracture union.(94) A systematic review by Corrales et al (95) reported eleven different criteria utilised to define union. Similarly, clinical trials indicate a lack of objective tools to assess fracture healing radiographically or clinically, making union as a nebulous primary outcome.(120)

Historically, radiographs have been a poor parameter and unreliable when used to define fracture healing. They have not been shown to be reliable or accurate when used to define union or determine the stage of healing.(121), (122) However, the

Radiographic Union Scale for the Tibia (RUST) has been shown to increase agreement among surgeons and radiologists in assessing fracture repair.(123), (124)

The RUST score is based on callus formation and the visibility of fracture lines at four cortices observed on anteroposterior and lateral radiographs. (Table 3-1) It applies a score from 1-3 to the medial, lateral, anterior and posterior cortex of the bone (total 12). (123), (124) One indicates a fracture line with no bridging callus. Two indicates a fracture line with bridging callus and three suggests no fracture line plus bridging callus.

Table 3-1. Overview of the RUST Score(124)

Score per cortex	Callus	Fracture line
1	Absent	Visible
2	Present	Visible
3	Present	Invisible

Since the RUST score was introduced into clinical practice, several studies have confirmed the overall interobserver agreement to be high between surgeons using this scoring system. (124), (125), (126), (127), (128), (129) Few studies have reported a value of the RUST score that corresponds to union. Based on clinical studies, Litrenta et al, McClelland et al and Fowler et al suggested that a minimum threshold for union on RUST score of 9, with Litrenta et al measuring it at 41 weeks (9month) post injury. (125), (127), (128) Therefore, any RUST score of 8 or less signifies a fracture has not united.

There has been debate on the most appropriate interpretation of the RUST score. Particularly deciding on a score 2 or 3.(129) The description in the original paper on whether a fracture line is still present has been interpreted in two different way.(124) Either there is no fracture line in the bridging callus or there is no fracture line in the cortex of the bone. Researchers have suggested that the fracture line should only

apply to the callus, and therefore, a callus which has a fracture line is scored as 2, while a callus which is bridged (i.e. no fracture line) is scored as 3. (124)

Computed tomography (CT) has been shown to be superior to plain radiography in assessment of union and visualisation of fracture lines.(130) However, this investigation is expensive and not easily available in the research setting. Using CT to assess union and non-union was not undertaken as standard management in the study sites of the research in this thesis.

Despite the advances in imaging, biomechanics and serology, physical exam remains one of the primary methods of determining fracture union in clinical practice, particularly in resource-limited settings, where radiographs or any other investigations may not be available. The lack of full weight-bearing is a very useful clinical diagnostic tool to assess fracture healing and is referred to as functional union.(131), (132)

There has been an increase in the use of patient reported outcome measurements (PROMs) in assessing fracture healing and as primary outcome measures in research studies, suggesting a shift towards patient-centric orthopaedic care.(120), (133), (134) PROMs assessments generally either measure general physical and psychological health, such as the EQ5D ((135), (136), or are disease-specific, such as the disability related index (DRI)(Appendix 13-1). (137), (138)

Eighty patients were studied using a generic quality of life PROM to evaluate changes in baseline score after treatment of long bone non-union fractures. (139) All patients with healed non-union demonstrated improved scores and decreased pain levels. This was seen to a greater degree in patients who achieved union by final follow up. These results suggest that it may be possible to track fracture healing via PROMs and future studies are needed to investigate the potential of using PROMs for diagnosis of non-union.(139)

3.7 Factors influencing bone healing

3.7.1 Age

In skeletally mature adults it has been suggested that advancing age has a significant impact on skeletal repair. (140) Studies of fracture healing in rats have shown that the formation of cartilage and bone, and cartilage resorption, were delayed in elderly animals.(141) Furthermore, there was evidence that accretion of mineral into the callus was reduced in elderly animals.(142), (143) In contrast, evidence from clinical research is conflicting and no valid conclusions can be drawn to date.(144), (145)

Clinical data is currently conflicting. Mills et al demonstrated, in a prospective cross-sectional study over a five-year period and involving just under 5,000 non-unions, that although the number of fractures increased with age, the number of non-unions did not. The non-union rate per fracture was highest in the 30 to 44-year age group, 20 times greater than that in the 0 to 14-year-olds and 2.5 times higher than in those aged over 75.(116)

3.7.2 Sex

Female patients over 55 years of age have demonstrated comparatively poor healing outcomes compared to the general population and a potential increase in non-union rate when compared to males.(59) After the menopause, women have a lower rate of oestrogen, which plays an important role in promoting bone formation, stimulating anabolic and reducing catabolic processes. Calori et al (59) suggest that these decreased oestrogen levels and generally diminished biologic activity may be responsible for the observed trends.(59) There is little evidence of any clinical difference in fracture healing in young (<55 years) adult male and females. However, there is a higher incidence of high energy injuries in young adult males, therefore

potentially increasing their risk of issues with union but this is speculative and requires further research.(116)

3.7.3 Diet

During the process of fracture healing, there is an increase in metabolism requirements in the body. For this reason, the importance of a diet rich in protein, calcium and phosphorus, and vitamin D has been assessed in a number of clinical trials.(146) Nutritional deficiencies seem to have the maximum influence on the later phases of bone callus formation.(146)

In an animal study, vitamin B6-deficiency caused a significant delay in the maturation of callus in rats.(147) Vitamin C has also been shown to be essential for the maintenance of differentiated functions of osteoblasts, including fracture repair, (148) and other investigators have shown that supplementary vitamin C in an animal model accelerates fracture healing.(149) Additionally, vitamin C content in the diet improved mechanical and histological parameters of fracture repair in a rat model.(149) Other researchers have shown the importance of dietary protein and calcium, phosphorous and vitamin D in fracture healing, again in an rat animal model.(146)

Despite increasing evidence in animal models, the lack of clear clinical research makes it difficult to draw conclusions regarding the exact effect of diet and nutrition on fracture healing in clinical practice.

There are also conflicting reports regarding high body mass index (BMI) on fracture healing. Some researchers suggest high BMI is a risk factor for non-union following fracture surgery (150), (151), whereas others report either no association between

union and high BMI (152), or an increase in surgical complications in obesity but not an increase in the rates of delayed or non-union.(153)

3.7.4 Vitamin D

At a cellular level, vitamin D is involved in every stage of the complex process of fracture healing through its effects on inflammatory cells, cytokines, growth factors, osteoblasts, osteoclasts and through its effect on the process of mineralisation. The role of vitamin D in fracture healing poorly understood. There are a limited number of conflicting experimental studies reporting either a negative (146), (154), (155) or no effect of vitamin D deficiency on fracture callus formation and mechanical callus quality. It is also debated whether vitamin D supplementation supports the fracture-healing process,(146), (156), (157), (158) although 70% of fracture patients display vitamin D deficiency.(159)

In rat models, vitamin D has been shown to be associated with impaired fracture healing compared to normal fracture healing in a vitamin D-sufficient control group. (160) Furthermore, animal models have demonstrated less resistance to torsional stress, (146) increased bone fragility, delayed union, smaller amount of callus and undermineralised bone. (146), (160) Fischer et al (158) demonstrated that fracture healing was only marginally disturbed in calcium and vitamin D-deficient mice. However, deficient mice displayed significantly increased serum parathyroid hormone levels and osteoclast activity, as well as reduced bone mass in the intact skeleton post-fracture, suggesting considerably enhanced calcium mobilisation from the intact skeleton during bone regeneration. Calcium and vitamin D supplementation initiated post-fracture prevented post-traumatic bone loss by reducing bone resorption and furthermore improved bone repair. These results suggest that adequate calcium and vitamin D supply post-fracture is essential to provide sufficient calcium for callus mineralisation in order to prevent post-traumatic

bone loss and to reduce the risk of secondary fractures in osteopenic patients with calcium/vitamin D deficiency.(158)

Regarding vitamin D's effect on BMD, a meta-analysis concluded there was very little evidence of an overall benefit of vitamin D supplementation on BMD.(161) Although small increases in bone density at some skeletal sites in some studies were reported, when these increases are offset against the individual findings of deleterious effects, the number of positive results is little better than what would have been expected by chance.(161)

3.7.5 Smoking

There is evidence reporting increase risk of delayed or non-union in people who smoke.(162) Nicotine prevents cellular proliferation during the fracture healing process, altering the maturing of macrophages and fibroblasts and acting directly on osteoblasts.(163) It is also a vasoconstrictor agent, causing an alteration of the tissue perfusion with consequent hypoxia and ischemia. A deficit in the formation of the haematomas at the fracture site and an alteration of biomechanical properties in the newly-formed bone has been assessed in patients who smoke more than 10 cigarettes per day.(163) Decreased vascularisation of a fracture site has also been reported to cause delayed bone healing leading to non-union.(164)

3.7.6 Alcohol

Alcohol has been shown to plays a role in inhibiting fracture healing, especially when it is taken in excessive doses in the post-trauma period.(165) Alcohol abuse in patients with fractures inhibits new bone formation and is considered to be associated with an increased incidence of delayed union.(165) The newly-formed

bone is lacking mineralisation and consequently has decreased mechanical stability at the fracture site due to low rigidity of the newly-formed bone.(165)

3.7.7 Medical comorbidities

3.7.7.1 Diabetes

Both animal and clinical studies have demonstrated a significantly higher incidence of delayed union, non-union, and an increase in fracture healing time in diabetic compared with non-diabetic patients.(166), (167), (168), (169) There are several implicated factors – mainly vascular and neuropathy problems. A reduction in the formation of collagen in the bone callus and a marked reduction of cells involved in the repair process have been noted in diabetic patients.(170)

Aderinto et al (171) reviewed 27 diabetic patients who sustained a tibial fracture treated with a reamed intramedullary nail and compared them with a control group who did not have diabetes. When reviewing the union rate, the diabetic patients had a non-union rate of 9%, compared to 0% in the control group.

3.7.7.2 Osteoporosis

Patients with osteoporosis experience a progressive loss of bone mass and an increase in the risk of fractures. Osteoporotic bone is bone that has an altered structure due to the reduced presence of trabecular components, which causes the loss of mechanical resistance. There is a reduction of osteoblasts and, thus, to callus production.(172)

The mechanical and biological factors that are involved in the healing process of bone are certainly affected by age and osteoporosis. Alterations in bone metabolism, like osteoporosis, seem to delay callus maturation and consequently decelerate fracture

healing. However, the mechanics of this influence of osteoporosis on fracture healing have not yet been clarified and clinical evidence is still lacking.(173)

3.7.7.3 Anaemia

Studies in iron-deficient anaemic rats have demonstrated significant deficiencies in bone healing, with a decrease in the rate of union and loss of strength. These changes have been attributed to a decrease in oxygen tension and a deficiency of iron, which is required for function of the electron transport system within the cell and for hydroxylation of proline in collagen formation.(174), (175)

Further work in a rabbit model showed an inhibition of fracture healing in hypovolaemia, which was attributed to impaired delivery of oxygen to the fracture site. These investigators found that a decrease in blood volume associated with anaemia delayed healing, but normovolaemic anaemia had no adverse effect, suggesting that attention to fluid rehydration following trauma was sufficient and that blood transfusion was not required to maintain normal fracture healing.(176), (177) Therefore, it appears that fluid resuscitation is important in the acute phase to allow fracture repair to progress normally, while attention to correction of chronic iron deficiency is necessary to decrease the rate of non-union: an important point to consider when managing polytrauma patients.

3.7.7.4 Peripheral vascular disease

Peripheral vascular disease adversely affects the blood flow to the tissues, including the bone and the surrounding soft tissue envelope. This will impair delivery of oxygen, inflammatory cells and nutrients to the fracture site. There will be an accumulation of carbon dioxide (CO2) and other metabolites rendering the local environment acidic. This combination of factors is considered to be detrimental to fracture repair. Although the correlation between peripheral vascular disease and

non-union has not been directly addressed, investigation of tibial fractures has shown that those with an associated injury to the posterior tibial artery have a significantly higher rate of non-union and take longer to achieve union than fractures without this vascular injury.(178), (179)

3.7.7.5 Hypothyroidism

The effect of thyroxine deficiency has been examined in a rat model of fracture healing in which methimazole was used to obliterate thyroid function.(180) This demonstrated that hypothyroidism inhibited endochondral ossification, resulting in an impairment of repair. Treatment with L-thyroxine returned the repair process to normal, suggesting that hypothyroidism will inhibit secondary bone healing, although primary bone healing appears to be unaffected. Again, it is not known if this effect translates to clinical practice.

3.7.7.6 Renal disease

Animal models have shown that progressive deterioration in kidney function leads to lower bone regeneration capacity following a fracture.(181) In addition, kidney disease results in the development of osteoporosis and an increase in fracture risk.(182) Therefore, due to these changes it is suggested that there is an increased risk in delayed and non-union in patients with chronic kidney disorders. But this is yet to be proven in clinical practice.

3.7.8 Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly avoided by orthopaedic surgeons because of their possible influence on bone healing. This view originates from multiple studies, in particular animal studies, that show delayed bone healing or non-union associated with NSAID exposure.(183) NSAIDs have been shown

to play a role in the reduction of osteoblastic activity and inhibit synthesis of prostaglandins, which in turn causes a delay in the formation of bone callus.(184) However, the true effect of NSAIDs on bone is still not known and the literature still does not offer a clear consensus with regard to the safety or harm of NSAID utilisation following orthopaedic procedures in clinical practice. The great variability in the interpretation of the available evidence appears to suggest that NSAIDs may affect bone healing, but that this effect depends on the type, dose, timing and length of exposure.(183), (185)

3.7.9 Injury, location of fracture and classification

There are number of factors to consider related to the injury and mechanism of injury that are likely to contribute to issues with the fracture healing process. The mechanical environment surrounding the fracture can affect the healing process. This is highly dependent on the fracture characteristics and the fixation technique utilised. Perren's theory of interfragmentary strain postulates that reparative tissue will develop at a fracture site in accordance to the strain tolerance of the tissue and the local strain environment between fracture fragments.(186) According to this theory, a simple fracture line that is not compressed and neutralised will have a higher likelihood for non-union relative to a fracture site exposed to a low strain environment. Some technical surgical factors can also affect union, for example, reamed femoral nailing reports a higher union rate than unreamed femoral nailing.(187) Furthermore, presence of a fracture gap has been indicated to increase the non-union risk; however, this variable must be taken in context with the fracture type (simple versus comminuted) and fixation strategy (compression plating or nail, bridge plating or external fixator).(188)

3.7.10 Mechanism

3.7.10.1 Low energy

Low energy injuries generally cause less significant damage to the soft tissues, and to the bone. The relatively minimal fracture site haematoma, the more stable fracture configuration and the minimal damage to the soft tissues and the periosteum rarely leads to healing delays and complications.(59) However, if a fracture is sustained with a low energy injury, it is more likely to be a fragility fracture and, as discussed earlier, there has been a suggestion that osteoporotic fracture may be associated with delayed bone healing.(173)

3.7.10.2 High energy

High energy injuries cause more complex, more comminuted and displaced fractures, often with considerable tissue loss, serious damage to the soft tissues and to the vascular system, compared to closed injuries. Therefore, these factors may lead to a reduction of blood flow to the fracture site and decrease the physiological inversion of the endosteal flow.(78)

Experimental studies have shown an up to 50% reduction of the cortical blood flow immediately after trauma due to vasoconstriction in the marrow and periosteum vessels.(189) In cases where the bony fragments are devascularised, or where periosteal stripping and destruction produces necrotic bony fragments and large defects, the risk of an atrophic non-vital non-union is high. It has been shown that serious damage to one or more important veins in the lower limb increases the incidence of non-union by about threefold.(190) Finally, fractures caused by high energy trauma are more difficult to reduce and stabilise compared to lower energy injuries. Therefore, potentially negative effecting bone union outcomes.(59)

3.7.11 Topography of fracture

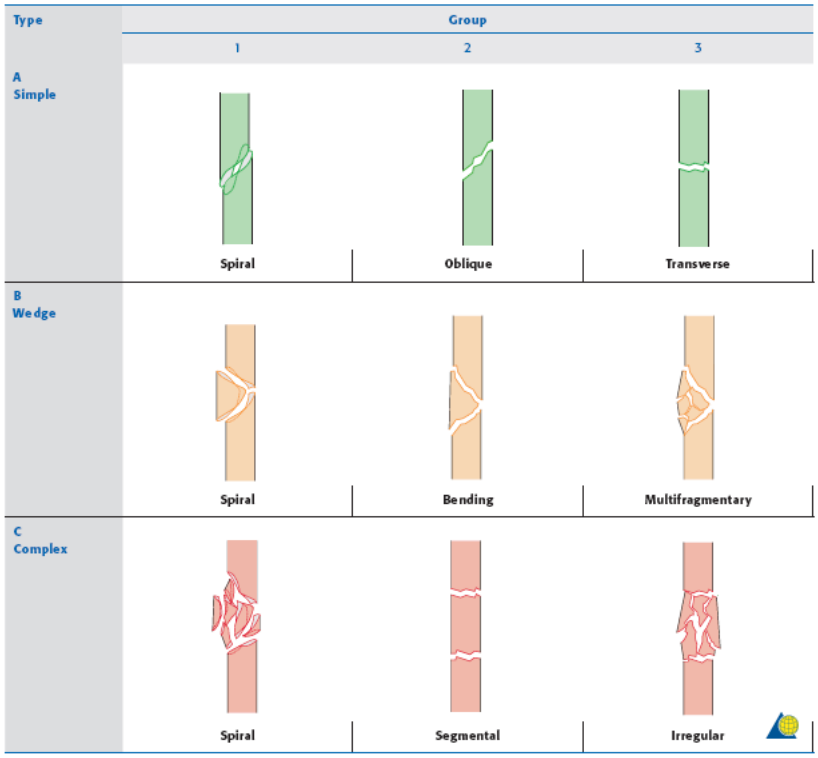
Fractures of the metaphysis area of a bone have a lower incidence of union defects and shorter healing times compared to fractures of the diaphysis.(59) The repair mechanism of metaphyseal fractures starts from cancellous bone that regenerates more rapidly and also has greater vascularisation. Healing processes in the metaphyseal area are therefore faster and less susceptible to obstructive factors than those encountered by diaphyseal fractures. (59)

Bone ossification can occur even when there is considerable damage to the periosteum and to the vascularity of the fracture fragments.(191) The anatomical site of the fracture compared to the location of the vessels that supply the bone is also important. Fractures that are distal to the nutritional centre have a greater risk of association with impaired blood supply and therefore a greater risk of developing an atrophic non-union.(192)

3.7.12 Classification of fracture

There are a number of ways to classify fractures. The most generic common classification is the AO classification by Muller.(193) Muller identified four different groups of fractures: simple, unstable, potentially unstable and highly unstable. The full classification system can be seen in Table 3-2.

Table 3-2. AO classification of fractures (189)



3.7.12.1 Simple - stable

Simple fractures, types A2 (oblique), A3 (transverse) and B2 (with an angulated wedge) have a single site or a wedge, potentially stable, that has a sufficient support to allow inter-fragmentary compression and fracture healing.

3.7.12.2 Complex - unstable

Complex fractures, types A1 (spiral), B1 (spiral wedge) and B3 (multi-fragmentary wedge) are potentially unstable, as they do not have a sufficient support area to guarantee fixation by simple inter-fragmentary compression.(194)

3.7.12.3 Comminuted - highly unstable

In comminuted fractures types C1 (spiral complex) and C3 (multiple fragments) the multiple fragments and comminution represent a high risk of devascularisation and necrosis of the fragments, with a consequent loss of substance and the need to provide a biological stimulus, in addition to mechanical stimulus, to obtain a good level of healing. Comminuted fractures can result in challenges with regards to stabilisation of a fracture, since it is not always possible to obtain a stable fixation that will facilitate callus formation and maturation.(195)

3.7.12.4 Segmental - potentially unstable

Only type C2 (two segments) belong to this group, which have a two fracture sites on the same bone segment. This again results in challenges related to fixation. On occasions, it is possible to obtain good level of healing of one of the two fracture sites, while the other one evolves towards non-union.(193) This group will evolve towards a type of non-union that differs depending on the type that each fracture site in the segment involved belongs to. It is type C fractures that have the greatest tendency to evolve into non-union: literature shows about 40% of type C fractures of the diaphysis of the femur evolve towards non-union, while types B and A have much lower percentages of incidence, 15% and 6% respectively.(196)

3.7.13 Open fractures

Certain characteristics of fractures can influence the progression of fracture healing. Disruption of the soft tissue envelope through either an open fracture (197) or open reduction during intramedullary nailing (198) has been shown to increase the risk of non-union. The degree of fracture comminution has also been shown to increase the risk of non-union in open fractures, likely due to substantial damage of the periosteum and soft tissue at the fracture site.(196)

The Gustilo-Anderson (GA) classification (Table 3-3), devised in 1976, divides exposed fractures into three groups that do not have a direct correlation with the evolution into non-union. (199) Group three were usually high-energy open fractures and were further subclassified by Gustilo et al into A, B, and C according to the severity of the soft tissue injury, and need for vascular reconstruction (Table 3-4).(200)

Table 3-3. Gustilo-Anderson classification system (199)

Type	Description
I	Skin wound less than 1cm Clean Simple fracture pattern
II	Skin wound more than 1cm Soft tissue damage not extensive No flaps or avulsions Simple fracture pattern
III	High energy injury involving extensive tissue damage Or multi-fragmentary fracture, segmental fracture, or bone loss irrespective of size of skin wound Or severe crush injury Or vascular injury requiring repair Or severe contamination including farmyard injuries

Table 3-4. Additional classification parameters added to the original Gustilo-Anderson classification (200)

Type	Description
IIIA	Adequate soft tissue cover of bone despite extensive soft tissue damage
IIIB	Extensive soft tissue injury with periosteal stripping and bone exposure Major wound contamination
IIIC	Open fracture with arterial injury requiring repair

The literature reports that grade II and III open fractures have a greater predisposition to non-union compared to grade I and closed injuries. This is due to the fact that they are related to high energy trauma with considerable necrosis of the soft tissues.(201) Severe fragmentations of the bone together with damage to the periosteal circulation reduces further the availability of osteogenetic factors at the

fracture site and increases significantly the rates of necrosis and atrophic non-union.(201)

3.7.14 Polytrauma

A commonly neglected factor which worsens the prognosis of a fracture is the coexistence of multiple trauma.(202) The most frequently used evaluation system for a multi-trauma patient is the Injury Severity Score (ISS).(203) The multiple trauma is generally considered as a systemic illness with various levels of involvement of both the musculoskeletal system and the internal organs. On the basis of damage to bones, which may or may not be associated with injuries to other organs, its considered that a multiple trauma patient are those with an ISS≥ 16.(203) However, patients with an ISS ≤ 16 but with more than one fracture could be considered as multiple fracture patients.

The incidence of non-union is significantly higher in polytrauma patients with multiple injuries (not just fractures) than in patients with isolated injuries.(204) Impaired bone regeneration in multi-trauma patients may be caused by several local changes that occur after high-energy impact, such as open fractures, poor condition of the surrounding soft tissue, and large-bone defects.(204)

Animal studies have suggested that systemic changes after multi-trauma could disturb fracture healing.(205), (206), (207) A animal study demonstrated that blunt chest injury altered the cellular composition of the fracture haematoma in rats and negatively affected the outcome of bone repair by inducing hypertrophic callus formation.(208) Also, intraperitoneal injection of lipopolysaccharides, a frequently used model that mimics trauma-induced systemic immune responses, disturbed fracture healing in rats by inducing hypertrophic callus formation.(209)

In a clinical study, Bastian investigated the relationship between the systemic immune response to severe injury and outcome of bone regeneration. They demonstrated that peripheral blood-leukocyte kinetics differed significantly between multi-trauma patients with normal and impaired fracture healing of the tibia during the first two weeks after injury. They hypothesised that systemic inflammatory changes after major trauma contribute to this high incidence of impaired bone healing in severely injured individuals.(209)

3.7.15 Infection

In a high proportion of non-unions, up to 40%, have undiagnosed underlying infection. (210) However, an infection does not necessarily result in non-union. It may contribute to the creation of conditions such as sequestrum (necrotic cortical bone), infected tissue, interposition of necrotic soft tissue between the fracture fragments and to hardware loosening and fixation failure.(211) Additionally, infections are more common following open fractures and are thought to contribute to a worse prognosis with high non-union rates. (59), (212)

CHAPTER 4. HUMAN IMMUNODEFICENCY VIRUS AND BONE

4.1 Aim of this chapter

In this chapter theories and mechanisms through which HIV has an influence on bone metabolism, BMD and blood supply to bone will be explored. Furthermore, the effects of HIV on a patient's fracture risk and their level of circulating vitamin D will be reviewed.

4.2 Overview

The key aspects involved in the relationship between fracture healing and HIV infection relate;
(a) to the effects of the virus and ART on bone metabolism, and
(b) to the effects of both the virus and ART on the immune system.

Altered rates of bone formation and resorption, in a disrupted metabolic and cytokine environment, not only have implications for the BMD and fracture risk, but also for remodelling in secondary bone healing.(19) A major factor known to affect fracture healing is local blood flow to the site of the injury. It is now well established that any stage of HIV infection is associated with osteonecrosis, due to interruption in osseous blood supply, although the mechanism for this has not been elucidated. (12),(14) ART has also been reported to contribute to this effect on the blood supply to bone.(19) Conditions that jeopardise arterial flow to the site of primary bone healing are associated with higher rates of delayed fracture healing and non-union.(89) Furthermore, a small number of clinical studies have investigated the role of HIV in the fracture healing process. (12),(14) These have suggested that HIV and/or ART are associated with delayed fracture healing and may result in non-union.(18), (213) Overall, the molecular and cellular mechanisms driving this remain unclear and the true effect of HIV and ART on bone healing is very poorly understood. Here a discussion of the suggested potential basic science mechanism by which HIV may influence bone metabolism will be presented and the influence of ART on bone.

In order to understand the potential mechanisms through which HIV and ART affect bone metabolism, bone formation and BMD, it is essential to have an awareness of the cells, factors and proteins involved in homeostatic bone remodeling. Osteoclasts form from precursors that derive from the monocytic lineage and express the surface molecule, receptor activator of NFk B (RANK). Under the influence of the key osteoclastogenic cytokine RANK Ligand (RANKL) these cells differentiate into bone resorbing osteoclasts.(214) The osteoclastogenic and pro-resorptive activity of RANKL is moderated by its physiological decoy receptor osteoprotegerin (OPG). In both humans and animals, the ratio of RANKL to OPG is considered to be the final arbiter of the rate of osteoclastic bone resorption, and an inappropriate balance between these two factors is a key factor in the bone loss associated with numerous skeletal diseases.(214)

4.3 Pathogenesis

4.3.1 TNF-α, RANKL and OPG

TNF-α plays a critical role in HIV pathogenesis and HIV utilises the TNF-alpha signalling pathway for spreading the infection.(215) TNF-α has been observed at high levels in HIV patients, (215) and high HIV ribonucleic acid (RNA) viral load and T-cell activation have been associated with elevated levels of RANKL, which may therefore lead to osteoclast formation and increased bone turnover.(216), (217), (218) Interferon gamma (IFNg), a physiological inhibitor of RANKL signalling, is considerably downregulated in advanced HIV infection.(214) Therefore, a limited capacity to suppress RANKL during HIV infection may also lead to increased osteoclast activation and bone resorption. TNF-α has also been shown to mediate apoptosis of human osteoblasts.(219) Additionally, Vikulina et al has shown that bone loss in HIV transgenic rats was associated with an increase in RANKL and a parallel decline in OPG levels, thus leading to increased overall osteoclastic bone resorption.(220)

4.3.2 Wnt signalling pathway

The lipoprotein receptor-related protein (LRP5) gene role is to provide instructions for making a protein that is embedded in the outer membrane of many types of cells in the human body. It is known as a co-receptor because it works with another receptor protein, frizzled-4 (produced from the FZD4 gene) to transmit chemical signals from outside the cell to the cell's nucleus. FDZ4 and the LRP5 protein participate in the Wnt signalling pathway, a series of steps that affect the way cells and tissues develop. Wnt signalling is important for cell division, attachment of cells to one another, cell movement and many other cellular activities.(221)

The LRP5 gene plays a role in the development and maintenance of bone, helping to regulate BMD.(221) The circulating factor Wnt binds to LRP5 on osteoblast surface membranes to initiate intra-cellular signalling. This drives production of osteogenic molecules including bone morphogenetic protein (BMP) and OPG, culminating in bone formation. It has recently been demonstrated that Dkk-1 (Dickkopf-related protein), a molecule which inhibits this pathway, and therefore bone formation, is induced by TNF-α and up-regulated in HIV-positive patients.(222)

4.3.3 Osteocalcin

Levels of osteocalcin are linked to the modulation of bone growth, bone mineralisation and have been used to show bone formation.(223) Osteocalcin has been shown to be reduced in the serum of HIV-positive individuals, and this has been reported to be a result of the down-regulation of calcitropic hormones parathyroid hormone (PTH) and 1,25 dihydroxychole-calciferal. Circulating osteocalcin has been shown to correlate positively with the CD4 count, and negatively with both TNF-α and TNF receptor,(224)

4.3.4 Inflammatory cytokines

The initiation of fracture healing is stimulated by local release of pro-inflammatory cytokines such as TNF-α, interleukin-1 (IL-1) and IL-6 activates cytokine cascades which starts the recruitment and differentiation of cells involved in the formation of fracture callus and bone healing. Inhibition of an inflammatory response by cyclo-oxygenase inhibitors is known to adversely affect the process of fracture healing.(225) Although HIV infection is immunosuppressive, as mentioned earlier, cross-sectional studies showing that serum TNF-α is often raised in HIV-positive subjects in comparison with healthy control groups indicate that the production of inflammatory cytokines is possible in the context of HIV.(224), (226) ,(227), (228), (229) The effects of chronically raised levels of TNF-α on fracture healing are speculative. Ongoing inflammation could prime the body for an inflammatory response to a fracture, resulting in a highly efficient up regulation of cytokines. Alternatively, an increased baseline level of TNF-α could theoretically lead to desensitisation, preventing or decelerating the healing process to the detriment of the patient.(19)

4.3.5 IGF-1

A study of perinatally HIV infected children has found an association between low concentrations of insulin-like growth factor-1 (IGF-1) and reduced bone ultrasound attenuation,(230) a correlate of BMD. An inverse correlation between IGF-1 and IL-6 was observed. In addition, IGFs have an anabolic function on the skeleton and IGF-1 is known to be the major effector of bone growth.(231) Both IGF-1 and IL-6 are involved in fracture healing, however their effect on fracture healing in advanced HIV infection is not known.

4.3.6 Immuno-skeletal interface

The immune and skeletal systems are closely interlinked and changes in the immune system influence skeletal metabolism. Osteoclast precursors are derived from monocytic cells and activated lymphocytes secrete osteoclastogenic cytokines including RANKL and TNF-α (232), and cytokines that drive up osteoclastic bone resorption in inflammatory conditions including rheumatoid arthritis (233), periodontitis (234), (235) and during oestrogen deficiency.(236) Additionally, in contrast, under basal physiological conditions, both human (237) and mouse (238) B cells are a source of OPG. B cell OPG production is further sustained by interactions with T cells, in part though CD40 ligand (CD40L) co-stimulation. (237), (238) Animal models of B-cell deficiency, T-cell deficiency and CD40 and CD40L deficiency all display severe bone loss and significantly diminished total OPG production.(238)

Interestingly, in HIV-transgenic rats there is a switch from the production of bone-sparing OPG by B cells to production of bone-destroying RANKL.(220) Furthermore, treatment of peripheral blood CD4 T cells in vitro with the HIV-1 coat protein gp120 caused a decline in T-cell OPG production. However, such actions have yet to be demonstrated in HIV-1-infected patients.

Another central cytokine involved in the immuno-skeletal interface is interferon gamma (IFNg). Under inflammatory conditions, IFNg has been found to promote bone loss by up-regulating the activity of antigen-presenting cells and leading to T-cell activation and osteoclastogenic cytokine production.(239) However, IFNg has also been shown to be a potent direct inhibitor of osteoclast formation by antagonising the downstream signal transduction from the RANKL receptor, RANK.(239), (240) Specifically, IFNg induces degradation of tumor necrosis factor receptor-associated factor-6 (TRAF-6) (240), a critical adapter protein that links RANK to downstream osteoclastogenic transcription factors including NF-k B and c-Jun N-terminal kinase. T helper-1 cells are a major source of IFNg and, Yadav et al (241)

recently reported that the suppressor of cytokine signalling-1 (SOCS-1), a potent inhibitor of IFNg signal transduction, is significantly elevated in cells derived from individuals with progressive HIV infection, as well as in HIV-1 transgenic rats. (240) It is speculated that up-regulated SOCS-1 may contribute to elevated osteoclastogenesis by removing the dampening effect of IFNg on the differentiation of osteoclast precursors in the context of HIV. (240)

The overall effect of T cells on bone, depends on their activation state. Activated CD4 T cells promote bone loss in inflammatory diseases such as rheumatoid arthritis(233) and periodontitis.(242) T cell–deficient mice have significantly increased bone resorption and reduced bone density, as compared to controls.(243) Conversely, resting $CD4^+$ T cells may contribute to dampening of bone resorption in vivo.(238) HIV is an inflammatory disease and therefore CD4 T cells could behave in a similar way as in a rheumatoid arthritis, resulting bone loss. Depending on the stage of the HIV infection and if an individual is on ART, this could influence activation of the CD4 T-cells and ultimately fracture healing.

Bone formation is inhibited by CD8 T cells and this has been demonstrated in a number of in vivo studies. (244), (245) In a mouse model, Reinke et al, established that depletion of CD8 T cells improved fracture callus formation and bone mineral density. Additionally, increasing the CD8 T cell population resulted in delayed fracture callus formation and decreased bone mineral density. (245) By affecting the function of CD4 T-cells and antigen presenting cells that are required for correct CD8 T-cell maturation, HIV is able to decrease the circulating pool of effector and memory CD8 T-cells that are able to combat viral infection. (244) The end result is a loss of CD8 T-cell function. This downregulation in CD8 T cells in HIV-positive individuals could theoretically result in a subsequent improvement in fracture healing, not seen in HIV-negative individuals with still normal levels of CD8 T cells. However, the overall action of T-cells and immune system on the fracture repair process in HIV-positive individuals is not fully understood.

4.4 Human Immunodeficiency Virus and bone mineral density

Osteoporosis is a significant global health problem that causes deterioration in the microstructure of the bone, leading to a compromised bone strength, which predisposes the individual to an increased risk of fractures of the hip, spine, forearm, and other skeletal sites. (173)

The diagnosis of osteoporosis is usually considered in the presence of a low-energy traumatic fracture and considered to be present when the bone density measurement (BMD) is found to be below a certain value. Dual energy X-ray absorptiometry (DEXA) is the gold standard of BMD and is measured at the lumbar spine and hip. Osteoporosis is defined as BMD measurement at the hip or spine ≤ 2.5 standard deviations below the mean BMD for a healthy, young, sex-matched population (T-score). Osteopenia is defined as a T-score between −1.0 and −2.5. Normal BMD is any T-score higher than -1.0.(246) A Z-score compares an individual's bone density to the average BMD of the population average of people with the same race, age and gender.

BMD can also be measured with peripheral imaging devices. These include the DXL Calscan (Demetch AB), a dual energy X-ray absorptiometry device for determining heel BMD. The system is based on the standard technique of dual energy X-ray absorptiometry, using a fan beam configuration, but introduces an additional laser measurement of heel thickness intended to improve accuracy. In a number of large population-based studies, when measuring calcaneal BMD with DXL, it has been confirmed that calcaneal BMD measurements appear to have predictive ability for osteoporosis-related fractures that does not substantially differ from that of gold standard measurements of lumbar spine and hip BMD using a standard DEXA machine. This has been shown in both male and females, old and young.(247), (248), (249), (250), (251) Furthermore, DXL measurement at the heel, using a T-score

threshold of -2.5 for classification of osteoporosis is in concordance with the WHO definition of osteoporosis. (250)

Measurement of BMD using a peripheral imaging device has a number of advantages over traditional BMD measurement with a standard DEXA machine. Practically it is simple to use, mobile and it is significantly quicker and cheaper to perform an assessment with compared to standard DEXA. For this reason, such imaging is now widely used in the NHS for screening of fragility fracture patients, instead of traditional measurements.

A reduction in BMD is a common complication of HIV and its treatment. This has been documented by a number of cross-sectional studies (8), (9), (10), (11), (252), (253), (254), with low BMD, osteopenia and osteoporosis being found in both male and female HIV-infected patients (255), (256) compared to non-infected individuals.(257) Furthermore, ART, independently has been shown to decrease BMD and this will be discussed in more depth in later chapter.(253)

A reduction in BMD is a well-recognised cause of fragility fractures (173) and, as such, HIV-related decrease of overall bone stock could potentially increase the risk of fracture and its related morbidities. With increasing survival attributable to better ART, an HIV-related reduction in BMD could compound age-related causes of altered bone density, such as the menopause, with clinical implications of an increase in fragility fractures worldwide in HIV-positive patients.(253) Furthermore, in the general population, it has been postulated that a reduced BMD is associated with a reduced speed of fracture healing, but this has not been investigated in HIV-positive individuals.(173)

4.5 Human Immunodeficiency Virus and osteonecrosis

One of the factors known to affect fracture healing is local blood flow to the site of the injury. (89) In experimental models, de-vascularised fracture sites have impaired healing.(15), (17) A further concern about the implications of HIV infection on fracture healing arises from the fact it is now established that HIV and its treatment can cause osteonecrosis, predominantly of the femoral head.(12), (254), (258), (259), (260)

Osteonecrosis is a condition in which there is cell death of various bone components including haematopoietic fat marrow and mineralised tissue due to reduced arteriolar blood supply.(12) The estimated incidence of osteonecrosis in the general population is 0.135%.(259) Risk factors for osteonecrosis, in the absence of HIV, can be subdivided into traumatic and non-traumatic. Traumatic causes include fractures and dislocations. Non-traumatic causes include alcohol abuse, collagen vascular disease, deep sea diving, systemic steroids, hyperlipidemia, hypercoagulability, sickle cell disease, smoking, Gaucher's disease, pregnancy, pancreatitis and irradiation.(12) However, in approximately in 8-20% of osteonecrosis cases, no association with the known risk factors has been noted.(261) The reported estimated incidence of osteonecrosis in HIV patients ranges from 0.45 to 1.33%, which is significantly greater than in the general population.(262), (263)

The reason for such a high incidence of osteonecrosis in HIV-positive individuals is not known. Some authors have attributed the increasing incidence to hypertriglyceridaemia, which is frequently seen in HIV-positive individuals. Hypertriglyceridaemia secondary to the use of protease inhibitors has also been reported.(261), (263), (13) Additionally, there have been associations reported between retrovirus infection and the presence of both raised anti-phospholipid antibodies (264) and protein-S deficiency.(265), (266), (267) This suggests the possibility of a microvascular, possibly microthrombotic, origin for circulatory

compromise. It remains unclear whether osteonecrosis is an HIV-related complication, an adverse effect of ART or is caused by another HIV-associated condition, as is the mechanism through which this occurs. However, conditions which may jeopardise arterial flow to the site of primary bone healing are associated with higher rates of delayed and non-union of fractures.(16) The suggestion that HIV infection may have adverse effects on blood flow, as evidenced by its reported association with osteonecrosis, may further contribute to problems in fracture healing.

4.6 Human Immunodeficiency Virus and fracture risk

There appears to be a concurrent increased fracture risk among HIV-positive individuals compared with HIV-negative populations. The National Osteoporosis Foundation has recently included HIV infection and ART as risk factors for osteoporosis and fragility fractures.(268) In a large population-based study of 8,525 HIV-infected and 2.2 million non-HIV-infected individuals, total fracture incidence was 2.87 (95% CI, 2.52–3.23) in HIV-positive individuals, compared with 1.77 (95% CI, 1.75–1.79) in the uninfected group (P<0.0001).(269) A nearly three-fold increase in incident fracture rates was identified among participants in the HIV Outpatient Study as compared to the National Hospital Ambulatory Medical Care Survey, a representative sample of the U.S. general population.(270)

A questionnaire-based evaluation of low-trauma fractures among Canadian women found that HIV-positive women were more likely to have a history of fragility fracture compared with women without HIV (OR, 1.7; 95% CI, 1.1-2.6) despite similar BMDs in both groups.(271)

More recently, a Spanish population-based cohort study including over 1.1 million participants and almost 2,500 HIV-infected patients showed a strong association

between HIV infection and hip fracture incidence, with an almost five-fold increased risk in the HIV-infected individuals, independent of sex, age, smoking, alcohol drinking and comorbidities. Similarly, a 75% higher risk of all clinical fractures and a 60% increase in risk of non-hip clinical fractures among patients with a diagnosis of HIV infection was found.(272) Finally, another nationwide case-control study in a Northern European population found that HIV infection is associated with an almost three-fold increase in overall fracture risk. In HIV patients with an almost nine-fold higher risk of hip fracture.(273)

4.7 Human Immunodeficiency Virus and vitamin D

The prevalence of vitamin D deficiency (defined as <35 nmol/L April through September or <25 nmol/L October through March) was reported as 29% among HIV-positive individuals in the Netherlands, Northern Europe.(274) Interestingly, vitamin D levels varied with ART medications, with lower levels occurring in NNRTI-treated compared with PI-treated individuals.(274) Tenofovir in particularly has been highlighted as the main ART contributing to vitamin D deficiency in HIV positive individuals.(275), (276)

In a study of HIV-positive individuals living in Boston, USA, the prevalence of vitamin D deficiency (defined as ≤20 ng/mL and >10 ng/mL) was similar to that listed in the study from the Netherlands, with 36.8% falling into vitamin deficiency group.(195) Approximately 10% of the study population in the Boston study were defined as having severe vitamin D deficiency (≤10 ng/mL).(195) A high proportion of low vitamin D stores (25[OH]vitamin D) in HIV-positive individuals as been reported.(277)

In summary, HIV-positive individuals are at risk of vitamin D deficiency and ART contributes to this. As discussed in Chapter 3, although vitamin D has been shown to have a role in fracture healing in animal models, the current available literature is too

scarce to confirm if this deficiency correlates with impaired fracture healing in the human patient population.

Note that throughout this thesis when referring to the measurement or value of vitamin D, this is reference to 25-hydroxyvitamin(25[OH]D.

CHAPTER 5. ANTI-RETROVIRAL THERAPY AND BONE: A Review of the Literature

5.1 Aim of the chapter

In this chapter the effect that ART drugs and combination therapies have on bone metabolism, BMD, fracture risk and fracture healing will be discussed. A summary of abbreviations for various ART drugs and drug combinations can be found in Table 5-1.

Table 5-1. Anti-retroviral therapies and abbreviations

NRTI	**Nucleoside reverse-transcriptase inhibitors**
ABC	Abacavir
AZT	Azidothymidine
FTC	Emtricitabine
3TC	Lamivudine
d4T	Stavudine
TDF	Tenofovir disoproxil fumarate
TAF	Tenofovir Alafenamide
ZDV	Zidovudine
NNRTI	**Non-nucleoside reverse-transcriptase inhibitors**
DOR	Doravine
EFV	Efavirenz
ETR	Etravirine
NVP	Nevirapine
RPV	Rilpivirine
PI	**Protease inhibitor**
APV	Amprenavir
ATV	Atazanavir
DRV	Darunavir
FPV	Fosamprenavir
IDV	Idiniavir
LPV	Lopinavir
NFV	Nelfinavir

RTV (referred to as r when in combination Lopinavir e.g. LPV/r	Ritonavir
SQV	Saquinavir
CCRT Antagonist	
MVC	Maraviroc
Fusion Inhibitors	
T-20	Enfuvirtide
Integrase inhibitor	**INI**
COBI	Corbicistat
DTG	Dolutegravir
EVG	Elvitegravir
RAL	Raltegravir

5.2 Overview

As mortality in patients with HIV began declining in 1996 following the introduction of combination ART treatment programs, there has been a shift in treatment goals from preventing death to prolonging life and improving health in individuals with HIV.(278) As discussed in Chapter 2, there are six classes of ART drugs. At the time the study was undertaken, the WHO recommends a combination of two NRTIs, with the NNTRI (TDF + 3TC (or FTC) + EFV) for the management of HIV.(50) This is the approach and regimen that is currently used in South Africa and is the therapy combination that the vast majority of patients currently under treatment for HIV in the Western Cape are taking. These guidelines are continuous evolving and being updated on an annual basis by the WHO. Patients are only given different treatment regimens in South Africa if they experience side effects to first-line therapy, are pregnant or an adolescent/child.

A number of ART drugs have been implicated in the emerging evidence that the therapy plays a role in HIV-associated bone disease. However, first-line therapies and regimens have evolved with development of newer medications, many with improved tolerance and dosing requirements. Accordingly, evaluating the direct effect of medications on bone has been difficult to assess long term.(278) Moreover, determining the direct effects of specific ART agents on bone health independent of their effects in suppressing HIV replication and associated inflammation has proven difficult. Finally, since they are taken in combination therapy regimens, determining the individual effect of one medication over another is not possible in clinical studies.

5.3 The effect of anti-retroviral therapy on bone and bone mineral density

In a meta-analysis comparing HIV-positive patients treated with ART to those who were ART-naïve, ART-treated individuals were more likely to have osteoporosis.(279) Across 11 studies of 884 HIV-positive patients, 67% had reduced BMD, of whom 15% had a osteoporosis, compared with HIV-negative controls. Compared with ART-naive patients (n = 202, 10 studies), ART-treated individuals (n = 824) had 2.5-fold increased odds of presenting with reduced BMD. However, it is important to note there was no adjustment for potential confounding factors in this study's statistical analysis. In other studies, ART duration has been associated with lower BMD in some groups.(280), (281), (282)

The Strategies for Management of Antiretroviral Therapy (SMART) study was undertaken to assess continuous ART treatment against intermittent ART to maximise ART benefits while minimising toxicities related to duration of ART treatment. However, the study was ended early due to an increased risk of death, AIDS-defining illness and other serious, end-organ disease in the treatment interruption group. Nonetheless, in individuals receiving continuous ART, loss of BMD was greater compared with patients taking intermittent ART.(283) In an additional analysis of the same study group, markers of bone turnover and inflammation were increased in patients on continuous ART, while intermittent ART was associated with an initial decrease in bone turnover followed by stabilisation.(284)

A number of studies have demonstrated that BMD may initially fall following ART initiation but recover after prolonged use.(285), (286) The majority of bone loss appears to occur in the first 12 months of starting ART. However, in a recent study of patients treated with ART for an average of five to eight years, there was significant continued bone loss on DEXA findings at the spine and hip. This loss was greatest among those individuals treated with TDF.(287) When trying to identify individual classes or ART medications of concern for bone health, BMD decrease seems to be

higher when initiating TDF (NRTI) and/or PI regimens, in comparison with NNRTIs. (288), (289), (290)

The AIDS Clinical Trials Group showed that the loss of BMD with ART initiation is greatest for those individuals with low CD4 cell counts, particularly those with a CD4 count < 50 cells/mm^3.(291) Additional factors that were independently associated with BMD loss included older age, female sex, lower BMI, higher HIV-1 plasma viral loads, initiation of PI and initiation of TDF.(291)

5.4 Nucleoside reverse transcriptase inhibitors – mechanism of action on bone

NRTIs inhibit the enzyme DNA polymerase-γ leading to loss of structure and function of mitochondrial DNA.(290) This, in turn, leads to alternative energy production, increasing lactate production from pyruvate, with resultant hyperlactataemia and even lactic acidosis in a smaller subset of patients.(290) In NRTI-treated patients, hyperlactataemia occurs in 15% to 20% of individuals, and lactic acidosis occurs in less than 0.4% patients.[10] It has been suggested that chronic acidosis induces calcium mobilisation from bones in an attempt to restore electrolyte homeostasis.(292), predominantly in trabecular bones, such as the vertebral bodies. In addition to metabolic changes, it is likely that NRTIs may have direct effects on osteoclasts. For example, ZDV has been shown to potentiate RANKL–mediated osteoclastogenesis.(293)

Starting TDF has been associated with increases in parathyroid hormone (PTH) levels, (294) which has been shown to enhance normal fracture healing in animal models, although this effect has not been shown to translate to the clinical setting.(295) Exposure of TDF to mouse osteoblasts *in vitro* demonstrated altered gene expression involved in cell signaling, cell cycle control and amino acid metabolism, implicating altered osteoblast function as a possible cause.(296) TDF may induce renal tubular

dysfunction, resulting in chronic phosphaturia, which may cause low total body phosphate despite normal serum phosphate, possibly leading to osteomalacia.(297), (298) Conversely, some studies do not corroborate the association between TDF and nephrotoxicity. In patients on TDF with a normal baseline renal function, no statistically significant hypophosphatemia was shown.(299) Furthermore, several additional studies have shown that the incidence of hypophosphatemia is the same between TDF-treated and untreated study groups.(300), (301) Overall, baseline renal function may be more important as a determinant of bone loss than the effects of TDF itself.

Inhibition of mitochondrial DNA replication occurs with some NRTIs, leading to osteoblast dysfunction.(302) Paradoxically, switching to TDF may improve mitochondrial function, suggesting that different ART drugs have varying effects on bone metabolism.(303) A further suggestion is that initiating ART causes a rapid recovery of T cells, peaking around 12 weeks. During immune reconstitution there are changes in circulating cytokine levels, including RANKL, potentially influencing bone loss.(284)

There are a number of different mechanisms through which it has been suggested the NRTIs may influence bone metabolism and BMD. A definitive mechanism has not been determined and the pathogenesis is poorly understood. It is likely that individual drugs influence bone metabolism through several pathways at once and to different overall extents.

This thesis will now go onto discuss studies investigating the individual NTRIs suspected of causing changes in bone metabolism and BMD.

5.4.1.1 Tenofovir disoproxil fumarate and bone mineral density

One of first ART studies to assess BMD investigated 600 ART-naïve patients randomised to receive either TDF or d4T, in combination with 3TC and EFV.(304) This demonstrated significant BMD reduction at 144 weeks with TDF compared to d4T treated groups at the lumbar spine (-2.2% vs. -1%, p=0.001) and hip (-2.8% vs. -2.4%, p=0.060). The majority of loss in the lumbar spine occurred within 24 weeks and stabilised after this point, with loss at the hip occurring within the first 48 weeks and then stabilizing thereafter. No further progression was noted at extended follow-up at 288 weeks.(305) Similar bone loss has been shown consistently in a number of trials of TDF.(306), (307), (308), (309)

In 220 Botswanans taking pre-exposure prophylaxis (PrEP) of TDF/FTC or placebo, use of TDF/FTC was associated with significantly less gain in BMD when compared with placebo at week 30 at the forearm (+0.97% vs. +1.83%, p=0.010), hip (-1.03% vs. +0.52%, p=0.003) and also the lumbar spine (-0.09% vs. +1.55%, p=0.001).(310) Significantly more participants had low BMD at baseline compared with that expected from study cohorts based high-income countries (6.8% vs. 2.3%, p<0.001). There were increases in BMD throughout the study in the placebo group, meaning that patients who were supposed to be healthy were, in fact, undergoing a period of normalisation of their BMD. However, consistently similar results have also been seen in a variety of populations, reinforcing the negative effect of TDF on HIV-negative individuals BMD.(311), (312)

BMD may also improve if TDF is switched to another NRTI therapy.(313) Finally, only one paper to date appears to show favorable effects of TDF on BMD.(314) Therefore, TDF does appear to cause an overall decrease in BMD, which may be time dependent with an initial loss that stabilises after around 24 weeks.

5.4.2 Other reverse transcriptase inhibitors and bone mineral density

Evidence of other NRTIs affecting bone metabolism and BMD is less clear and many trials use TDF as a comparison. Therefore, concluding that the medication is only less detrimental comparatively to TDF.

A randomised trial of 41 patients randomised to either continue LPV/r + 2NRTIs or switch to LPV/r + 3TC saw increases in total BMD in the switch group (+1.04%, 95% CI 0.06 to 2.01%).(315) No significant differences were seen in the continuation group and a larger increases in BMD were seen with discontinuation of TDF. Similar favourable effects of 3TC have been seen when switching from NRTIs to TDF/FTC or ABC/3TC and with switch from ABC/3TC to TDF/FTC.(316), (317), (318), (319)

A study of 24 patients randomized 3:2 to either halve their treatment dose of d4T or continue unchanged showed that at 48 weeks, half-dose treatment group stopped the progression of BMD loss (0.0% vs. -1.7%, p=0.003).(320) The authors suggested that the lower dose might be less tolerant to breaches in adherence, but full dose appeared more detrimental to bone.

In a trial of 50 men, BMD loss was greater in participants initiated on ZDV/3TC compared with NVP at the femoral neck (-6.3 vs. -2.3%, p=0.0006) and lumbar spine (-5.1 vs. -2.6%, p=0.07).(321) BMD changes with NVP stabilised after 12 months, whereas in the ZDV/3TC group, continued to deteriorate at the femoral neck for up to 24 months. ZDV/3TC has demonstrated similar effect on bone to TDF/FTC previously.(322) However, no studies to date has investigated the effect of TDF or another other NRTI on fracturing.

5.5 Protease inhibitors – mechanism of action on bone

PIs stop viral replication by selectively binding to viral proteases and then blocking proteolytic cleavage of protein precursors that are required for the production of infectious viral particles.

As discussed in Chapter 2, the Wnt/β-catenin pathway is involved in osteoblast differentiation and drives forward the production of osteogenic molecules including bone morphogenetic protein (BMP) and OPG, ending in subsequent bone formation. The PI RTV has been shown to increase the production of proteins that block the canonical Wnt β-catenin signalling. (323) RTV has also been shown to inhibit the pathway, potentially resulting in a decrease in osteoblast differentiation. Therefore, contributing to the pathogenesis of decreased BMD seen in patients on RTV.(324)

RTV has been shown *in vitro* to inhibit osteoclast differentiation directly in a dose-dependent manner. The effects were reversible within three days of removing RTV from *in vitro* cultures, with maintenance of normal osteoclast differentiation.(325) Confirming these *in vitro* findings, the ANRS 121 Hippocampe study group, demonstrated that reduction in lumbar spine BMD was significantly worse in the PI/r (boosted PI with RTV) and NNRTI arm (-4.4%±3.4%) and the PI/r and NRTI arm (-5.8% +/- 4.5%), compared with the NNRTI and NRTI arms without PIs (-1.5%±2.9%).(326)

RTV, but not IDV or NFV, has been shown to block interferon-γ-mediated TRAF6 degradation. TRAF6 has an important role in osteoclastogenesis, by acting as the intermediate between RANK activation and NF-κB and MAP kinases activation. This effect of RTVs on bone, appears to be reversible by interferon-γ administration. (323) Other *in vitro* studies have confirmed these initial findings that RTV has a role in inhibiting osteoclast cell differentiation.(325), (327) Further *in vitro* studies have demonstrated that osteoclast activity increases in the presence of NFV, IDV, RTV and SQV but not LPV and APV.(324) Whereas, other studies demonstrated that FPV

significantly increased OPG and decreased RANKL *in vivo*, but other PIs, including ATV, SQV, and IDV, did not.(328)

An *in vitro* study demonstrated that the PIs RTV, IDV and NFV decreased cytochrome p450 activity, in particular 25-hydroxylase and macrophage 1α-hydroxylase, resulting in decreased vitamin D.(329) However, clinical data linking PI with impairment of vitamin D are limited and overall inconsistent. Some studies have reported an association between RTV exposure and lower odds of vitamin D insufficiency or deficiency(330) and others have found higher levels of vitamin D in HIV-positive individuals taking PIs therapy compared with those taking NNRTI or naïve patients.(274)

Overall, there is a variation in the mechanism and extent to which different PIs influence bone metabolism, and these are still poorly understood.

5.5.1 Protease inhibitors and bone mineral density

A cross-sectional study of HIV-positive men taking PIs compared to HIV-negative individuals showed higher incidence of low BMD (relative risk (RR) 2.19 [95% CI 1.13 - 4.23] p-value = 0.02) in those taking PIs.(331) These results have been confirmed by additional research (256), (279), (332), and one research group demonstrated recovery of BMD after stopping PI therapy.(333)

When compared to other ART groups, conflicting results have been shown regarding the degree of BMD loss with different PIs. Patients initiated on PIs had significantly lower BMD at 48 weeks compared with NNRTIs and NRTIs (-4.4% vs. -1.5%, p=0.07 and -5.8% vs. -1.5%, p=0.001).(326) Conflicting reports comparing EFV (NNTRI) or LPV/r (combination PIs) with ZDV/3TC (NRTI) in 106 ART-naïve patients over 96 weeks reported significant decreases in BMD in both groups (p-value = 0.01).(334) However,

differences between groups were not significant (-2.3 vs. -2.5%, p=0.86). Another study compared three treatment regimens, LPV/r, EFV and 2NRTI, in paired combinations and reported similar results.(335) A similar trend has been shown by further studies comparing different treatment ART regimens with PIs. (331), (336), (337)

Interestingly, in a comparison of continuing TDF/FTC/EFV (NTRI and NNTRI combination) or switching to DRV/r (PI monotherapy), BMD at the femur increased with DRV/r (+2.9% vs. -0.003%, $p<0.05$) and also the lumbar spine (+2.6% vs. +0.008%, $p<0.05$).(338) This suggest that TDFs effect on BMD is greater than PI combination therapy. However, available results are conflicting, and this is likely due to the inherent nature of the effect of varying combinations of ART on bone, making it difficult to draw valid conclusions about individual ART drugs.

The data concerning PIs effect on BMD might also be explained by the increase in visceral fat.(339) It has been shown that there is a substantial gain in fat mass correlating with a sharp drop in BMD observed in individuals taking PI therapy.(340) Simulated increases in body fat have demonstrated reduction in the mean DEXA measurement of spine BMD, although not in the hip.(341) However, like with NRTI, no studies have investigated the effect of PIs on fracture healing to date.

5.6 Other anti-retroviral therapy and bone mineral density

Additional classes of ART, other than NRTIs, NNRTIs and PIs, have been shown to have an effect on bone and BMD. At the time this study was undertaken, these classes were not first line therapy in South Africa and few, if any, of the patients in this study cohort were taking any other classes of ART, other than NRTIs, NNRTIs and PIs. Therefore, they are not the focus of this thesis.

INIs block the action of integrase, a viral enzyme that inserts the viral genome into the DNA of the host cell. Because integration is a vital step in retroviral replication, blocking it ultimately stops further spread of the virus. Starting HIV treatment with RAL (INIs) + DRV/r (PIs combination) caused less BMD loss compared with TDF/FTC (NRTI combination) + DRV/r over 48 weeks (+9.2 vs. -7.0 g/cm^2, p=0.002).[72] This again suggests BMD loss is greater with TDF combinations. Unfortunately, virological efficacy was inferior with RAL, but its beneficial effect on bone when compared with NRTIs and PIs has been demonstrated in a number of other studies.(337), (342), (343), (344), (345), (346), (347)

In a cohort of 262 patients, those taking CCRT antagonist maraviroc had significantly higher BMD compared to those on TDF (-1.51% vs. -2.40%, p<0.001).(348) These results were echoed by the GUSTA trial, which compared switching to MVC+DRV/r against the continuation of stable ART.(349)

5.7 Emerging anti-retro viral therapy: Tenofovir Alafenamide

TAF is a pro-drug of TDF that has recently been licensed and may cause fewer side effects than TDF. A pro-drug is a biologically inactive compound that can be metabolized in the body to produce a drug. Regarding TAF, this is hydrolysed to TDF intra-cellularly by cathepsin A, resulting in higher intracellular tenofovir diphosphate with lower dosing.(350)

An *in vivo* study in dogs demonstrated preferential distribution of TDF to lymphatics with TAF compared with TDF.(351) The plasma levels of TDF were 10% less than those with TDF administration, which may reduce bone and other side effects of TDF. (351)

A number of clinical trials have compared the treatment of TAF with TDF using BMD as an outcome. All showing an improvement in BMD with treatment with TAF.(352),

(353), (354), (355) Two phase 3 clinical trials comparing EVG/COBI/FTC and TAF with EVG/COBI/FTC and TDF in 1733 patients over 48 weeks showed patients on TAF combination therapy had a reduction in changes in BMD at the hip and spine. They also experienced fewer side effects, with the exception of increases in total cholesterol, LDL and HDL cholesterol with TAF.(353) Similar results were found at 96-week follow-up, with BMD not changing.(354)

Recently, the reported beneficial effects of TAF compared to TDF have been questioned. A meta-analysis reported, from nine clinical trials, that TDF in combination with RTV or COBI was associated with higher risks of bone and renal adverse events, and lower HIV suppression rates, when compared with TAF. By contrast, when RTV and COBI were not used, there were no efficacy differences between TAF and TDF, and little differences in safety. The authors concluded that health economic value of TAF compared to the relatively low-cost generic TDF may be limited when these drugs are used without COBI AND RTV.(356)

5.8 Anti-retroviral therapy and fracture risk

As discussed, it has been well established that certain ARTs, particularly TDF and PIs, cause a decrease in BMD, although this varies between therapies. However, it is important to determine if this decrease in BMD translates to an increase in the risk of fragility fracture. In a recent publication from the Veterans Health Administrative Data Clinical Case Registry (VHA CCR), researchers characterised the risk of osteoporotic wrist, vertebral or hip fractures among a cohort of 56,600 HIV-positive individuals.(288) [32] Cumulative exposure to the ARTs TDF and LPV/r were both independently associated with fracture.

A large retrospective observational study of 56,660 patients determined that TDF had a fracture hazard ratio of 1.08 (95% confidence interval (CI) 1.02 - 1.15, p-value =

0.001).(288) Whereas, cumulative exposure to the antiretrovirals TDF and LPV/r were both independently associated with fracture.

Another recent publication evaluated fracture risk after ART initiation among 4640 HIV-infected individuals. (357) They identified 135 persons who experienced 151 incident fractures occurring a median 2.3 years after ART initiation. Fracture rates were significantly higher in the first two years after ART was started, when compared to subsequent time periods. The type of ART an individual was taking was not associated with fracture incidence. The authors speculate that BMD decline with ART initiation is linked with change in bone mass and quality, leading to increased fracture risk. Additionally, in theory, as patient health improves over time with ART, the risk of falls and subsequent fractures may decrease with overall health improvement, suggesting there may be a catabolic window after ART initiation that leaves individuals susceptible to a fragility fracture.

The most recent of studies investigating at fracture risk and ART found no evidence of any increase in risk of a fracture after exposure to TDF or to PIs. Among 861 reviewed cases, 261 fractures were osteoporotic and 254 of cases were matched to at least one control (376 controls). 49% of patients had been taking TDF and 82% had been on PIs. After taking into account a number of confounding factors, including the AIDS status, geographic location of an individual's home, body mass index, smoking status, alcohol consumption, exposure to steroids and the time point an individual was enrolled, there was no association between the risk of fracture and exposure to TDF (OR 1.04 [CI 0.86-1.27]), to NRTIs, or to PIs (OR 1.02 [0.92-1.12]).(339)

ARTs, particularly TDF and PIs, may increase the risk of an individual with HIV sustaining a fracture due to decrease in BMD; however, results are conflicting with the most recent evidence showing no excess risk of fracture after exposure to TDF or to PIs. As already highlighted, there is currently no literature available reporting the results of fracture healing in a large enough cohort of patients to analyse the effect

of ARTs on the fracture repair process. Therefore, no conclusion can be drawn to address the question of whether ART impairs fracture healing. The cohort reported in this thesis is the largest to date and will hopefully shed some light on this issue.

5.9 Summary

The treatment of HIV has evolved significantly since the approval of ZDV in 1987 for the treatment of HIV. The initial aim of ART was to slow a pandemic of HIV. Development of new ART drugs and administration of them in combination, with the aim of reducing resistance and improving efficacy, has resulted in the life expectancy of an appropriately treated HIV-positive individual may approach that of an HIV-negative member of the population.(358)

Combination therapy and the inability to compare HIV-infected patients with untreated controls makes it difficult to draw clear conclusions regarding the definite effect of different ART on bone BMD, bone metabolism, fracture risk and fracture healing. Evidence to date appears to implicate TDF as having the clearest detrimental effect on BMD, closely followed by various PIs. Other drugs in the NRTI class may have similar, but less severe effects. The effect of ART on fracture healing has not been previously investigated in laboratory or clinical studies.

It is unethical to use HIV-infected patients as non-treated controls, making it challenging to determine the exact degree of bone loss that is caused by ART on top of that caused by the HIV infection itself. In addition, it is difficult to draw conclusions from studies treating uninfected patients with ART as pre-exposure prophylaxis, as the demographics of such high-risk groups have not historically matched the general HIV-infected population. Comparison must be made between drug combinations, and drawing conclusions is often difficult.

Changing to ART with a favourable bone profile may lead to recovery in BMD, although this needs to be balanced with maintaining the virological efficacy of the alternative ART therapy, which is essentailly the primary reason for the treatment itself. Additionally, in the majority of studies, follow-up seldom exceeds 96 weeks in prospective trials and so it is currently unknown how patients' BMD is affected 10 or even 20 years later, or if fracture risk can be reduced in particularly at-risk patients by switching their ART when their BMD reaches a specific point. However, minimising BMD loss in this group of patients is particularly beneficial, as HIV itself may also reduce BMD, and patients with HIV may have other comorbidities that compound this effect (increased rate of smoking, poor nutrition). Nevertheless, the significance of a slight decrease in BMD that does not continue to decline remains to be proven, especially if an alternative ART may lead to relapse of the viral disease. Therefore, it is important that further research is undertaken taking into account these factors, to get a better understanding of the effect of ARTs and combinations on BMD, bone metabolism, fracture risk and fracture healing.

CHAPTER 6. FRACTURES IN HIV-POSITIVE INDIVIDUALS: A Systematic Review of the Literature

6.1 Aims of chapter

This chapter aims to report the findings from a systematic review of the outcomes of operative treatment of fractures in HIV-positive individuals. A formal meta-analysis could not be carried out due to the variability in the methodology and outcome measures in each study.

This systematic review has been published under the title 'Fracture management in HIV-positive individuals: a systematic review' in the journal 'International Orthopaedics'.(213) This chapter uses much of the information from this systematic review and the contribution by all the authors is acknowledged. For this publication, the author of this thesis (Simon Graham) devised the concept of the paper, undertook the literature search, lead the write up, edits and final submission of the paper.

6.2 Overview

A small number of clinical studies have investigated the role of HIV in the fracture healing process. These studies have suggested that HIV and/or ART may be associated with impaired fracture healing. (18), (19) The molecular and cellular mechanisms driving this remain have been discussed in previous chapters but main unclear and the effect of HIV and ART on bone healing is very poorly understood. This chapter assesses the current available clinical literature to date on the management of fractures in HIV-positive patients.

6.3 Methods

6.3.1 Data source and search strategy

The search strategy was made with key concepts identified using the population, intervention, comparator and outcome (PICO) process (359) to determine search-terms. These were expanded to include substitutes, related terms and alternative spellings. Medical subject headings (MeSH) were combined using a Boolean technique to improve the search.(360) Following this, specific terms and limitations were introduced and combined to refine the search (361) (Table 6-1). Both Scopus, PubMed and Web of Science databases were searched. The final search was carried out on 1st January 2018.

Table 6-1. MeSH Terms.

MeSH term 1	MeSH term 2	MeSH term 3
HIV	Deep surgical site infection	Infection
	Delayed union	Sepsis
	Early infection	Union
	Fractures	
	Implant sepsis	
	Late infection	
	Non-union	
	Surgical site infection	
	Pin site infection	
	Wound infection	

6.3.2 Selection criteria

The eligibility criteria are listed in Table 6-2

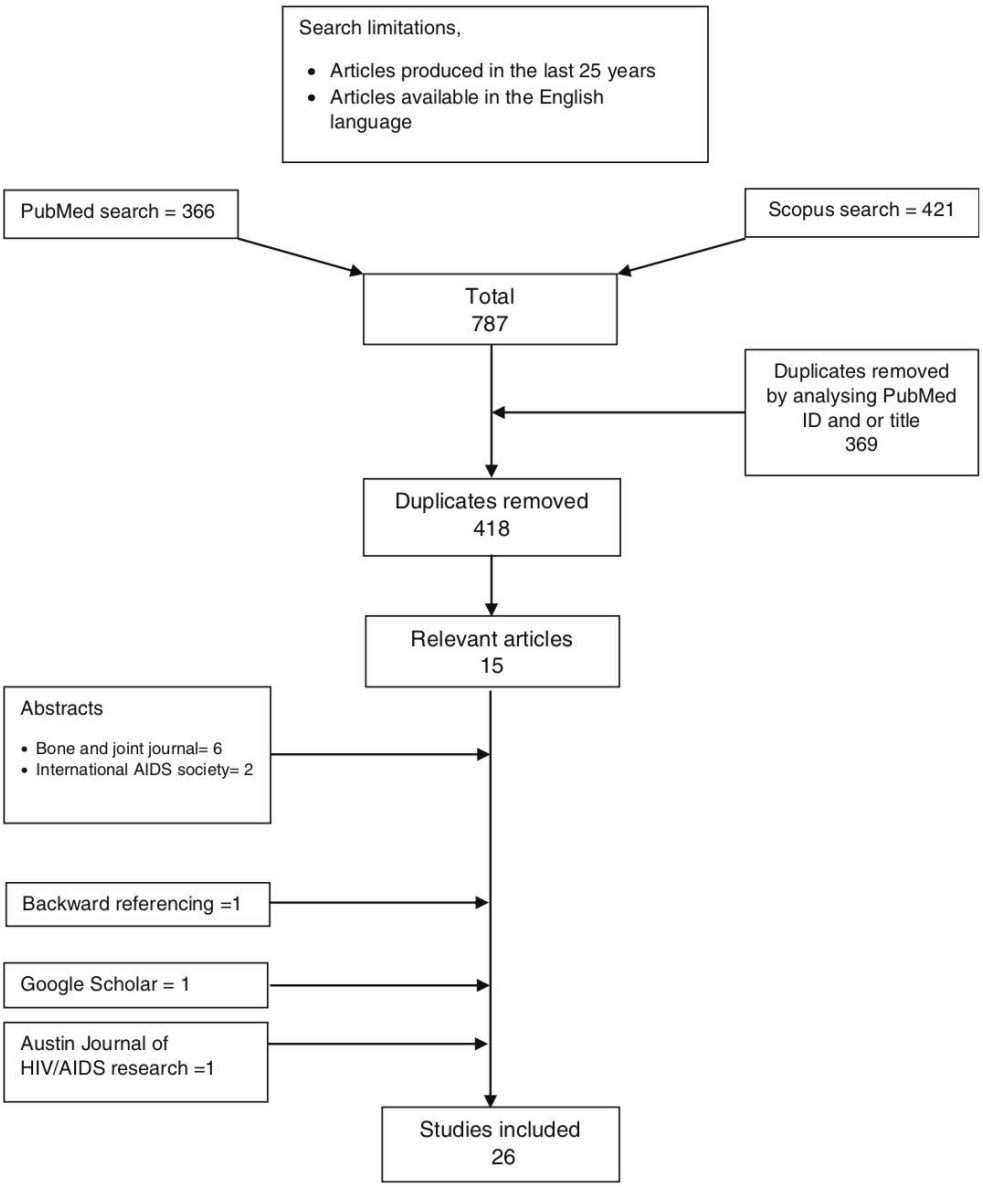
Using the studies identified, backward referencing of eligible studies and existing reviews was undertaken to increase the number of relevant studies. The abstracts of relevant orthopaedic and HIV/AIDS conferences were included to expand the number of studies included.

The systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance.(362) The process of the literature search is summarised in Table 6-2.

Table 6-2. Inclusion and exclusion criteria

Type of criteria	Description	Rationale for criterion
Inclusion Criteria	Short- or long-term patient outcomes and postoperative management of fractures in HIV or AIDS	As per research question.
	Where indication for surgery was not a fresh fracture, other procedures such as surgery for malunion, non-union and revision surgery were included as long as the majority of the procedures were fracture fixations.	This is to maximise the includible literature.
	If multiple articles from the same study were published more than once, only the single best study article was chosen unless subsequent publications included new data.	
	Studies undertaken in the last 25 years	
Exclusion Criteria	No numerical data presented for the HIV-positive category.	As per research question.
	If the complications were not categorised into open and closed fractures.	This is required to make a comparison of results among studies.
	Case reports.	Epidemiological evidence from case reports is not helpful.
	Languages other than English.	No resource available for translation.

Figure 6-1 Flow diagram of literature search (213)



6.3.3 Literature search

The final search identified a total of 26 studies, which were included in this systematic review. (Figure 6-1)

6.4 Results

6.4.1 Early Infection

6.4.1.1 Closed fractures

There were seven studies that investigated early wound infection and/or early implant sepsis in closed fractures managed with open reduction and internal fixation following a fracture (Table 6-3). The time period used to determine infection type varied between all of the studies. In this systematic review, early wound infection was defined as an ASEPSIS (363) score >10, as this would suggest a disturbance of wound healing.(363) This same definition of an ASEPSIS score of greater than 10 was used by the Malawi research group.(363) However, the definition used in the other studies was not consistent.(18), (364-369)

In a retrospective study, Paiement et al (364) (San Francisco, USA) reported a zero wound infection rate for closed fractures for HIV-positive patients (n=14), compared to a rate of 4% in the HIV-negative control group (n=446). As ART status was not included in the paper, and as this study was undertaken prior to the introduction of ART, it is assumed that none of the study participants were taking ART.

Harrison et al (18) (Malawi), reported similar findings in a prospective single blind study. They reported the results of 28 HIV-positive individuals that were not taking ART and a control HIV-negative group of 108. Wound infection rates were 4% and 6% respectively and this differences in the proportion of infections was not statistically significant.

Bahebeck et al (366) (Cameroon) demonstrated an infection rate of 5% (n=74) in HIV-positive participants compared to 1% (n=572) in the HIV-negative. This study prospectively analysed a cohort of patients with closed fractures that underwent surgery following a fresh fracture, and also non-union, malunion, aseptic necrosis and

osteoarthritis. Prior to surgery, 5% of participants were taking ART, which increased to 59% (n=74) at follow-up. Forty-four patients had a CD4 count <500 cells/mm^3.

In the largest prospective single blind study, researchers from Malawi reported the outcome of 118 HIV-positive cases and 418 HIV-negative controls undergoing fracture fixation surgery.(368) They reported wound infection rates of 4% in HIV-positive participants and 6% in the HIV-negative group, which was not statistically significant. Only 5% of the 118 HIV-positive patients were initially on ART, which later increased to 16% on follow-up.

Similar overall rates were reported by Nawale et al (365)(India) in a retrospective analysis of 35 ART-naïve HIV-positive participant's and 35 HIV-negative controls control. The wound infection rates were 6% (n=35) and 4% (n=35) respectively.

The most recent study carried out by Hao et al (369) (Denver, USA) did not use the ASEPSIS score to define infection. Instead, the surgical site infection (SSI) was used (Centre for Disease Control/National Healthcare Safety Network). In 24 patients with HIV, with 92% (22/24) taking ART at the time of injury, one participant developed an SSI, therefore a 4% rate of early wound infection was reported.

Not all researchers have found low rates of infection. Abalo et al (367) (Togo) reviewed HIV-positive patients with 28 closed fractures that underwent open reduction and internal fixation. They reported an infection rate of 29% in this study population and before surgery, 35% of the participants were on ART. There was no control group included in the study.

Overall, other than in one study (367), the evidence suggests that HIV is not a risk factor for early implant sepsis following fracture surgery

Table 6-3. Early infection in closed fractures

Author	Study type	Geographical location	Follow up period (months)	Patients on ART prior to study	Patients on ART at follow-up	Definition of early infection	Outcome Wound infection		HIV staging categories	Statistical analysis	Standard methods of evaluation	Limitations
							Study	Control				
G.D. Paiement (364)	RSB	USA (San Francisco)	6.5	0/14 [0%]	0/14 [0%]	30 days	0/14 [0%]	14/446 [4%]	-	P value = 0.035, Chi-square in HIV-positive group and wound infection.	CDC definition.	Retrospective.
W.J. Harrison (18)	PSB	Africa (Malawi)	3	0/28 [0%]	0/28 [0%]	Three months	1/28 [4%]	6/108 [6%]	WHO staging, Stage 0=2 [80%], Stage 1=3 [8%], Stage 2=2 [5%], Stage 3=2 [5%] Not known= 1 [2%]; CD4 cell counts >500=16%, 200-500=49% <200=35%	P value for closed fractures between HIV-positive vs HIV-negative = 0.396. Not significant.	ASEPSIS wound score >10.	-
S. Nawale (365)	R	Asia (India)	N/M	0/35 [0%]	0/35 [0%]	N/M	3/35 [9%]	2/35 [6%]	CD4 counts were between 250-500. Values not mentioned.	N/M.	ASEPSIS wound score.	Retrospective. Not blind.
J. Bahebeck (366)	P	Africa (Cameroon)	3	3/74 [5%]	44/74 [59%]	3 months	4/74 [5%]	3/572 [1%]	CD4 > 500=30 [41%], <500=44 [60%]	Fisher's exact test significant if <0 05, p value=0.87.	N/M	Not blinded, no standard use of scores to measure infection.

Author	Study type	Geographical location	Follow up period (months)	Patients on ART prior to study	Patients on ART at follow-up	Definition of early infection	Outcome Wound infection		HIV staging categories	Statistical analysis	Standard methods of evaluation	Limitations
							Study	Control				
A. Abalo (367)	R	Africa (Togo)	27	16/36 [44%]	16/36 [44%]	N/M	8/28 [29%]	-	CD4>500=21 [58%], 200-500=12 [33%], <200=3 [8%] Infection rates higher in symptomatic patients.	P value <0.05	N/M	No control. Retrospective. Not blinded. No standard methods of ASEPSIS score used to measure infection.
J. Bates (368)	PSB	Africa (Malawi)	1.5	7/139 [5%]	22/139 [16%]	Six weeks	5/118 [4%]	25/418 [6%]	CD4<500=27 [22%], 500-200=69 [56%], >500 = 28[23%] No relationship between ASEPSIS and CD4 count.	P value <0.05 significant, HIV-positive open vs HIV-negative p=0.064. Not significant.	ASEPSIS wound score >10.	Follow-up of only six weeks.
J. Hao (369)	P	USA (Denver)	12	22/24 [92%]	22/24 [92%]	Three months	1/24 [4%]	-	CD4>500=6 [28%], 200-500=10 [48%], <200=5 [24%]	N/M	SSI-CDC/NHSN classification.	High loss to follow-up. No control. Small sample size. Not blinded. Abstract only

Key: P - prospective study; PSB - prospective single blind study; R - retrospective study; RSB - retrospective single blind study

N/M - not mentioned

6.4.1.2 Open fractures

6.4.1.2.1 Wound infection

Fourteen studies examined wound infection in HIV-positive individuals managed operatively following an open fracture (Table 6-4). The study design was extremely heterogeneous these studies. A number of different definitions of wound infection were used and in some not stated at all. Varying fixation methods and injuries were included and commonly the grade of open injury was not always defined. When external fixators were used to manage injuries, it was not always possible to determine if authors were reporting wound infection rates regarding pin track infections or infection of the fracture. Furthermore, the majority of studies were retrospective, and study participants were followed up for different lengths of time.

Howard et al (370) (South Africa) studied open tibial fractures and showed an early wound infection rate of 11% (n=28) in their HIV-positive study population, compared to 20% (n=57) in the HIV-negative controls. In the HIV-positive participants, the mean CD4 count was 432 and 11% (n=28) were on ART. In a prospective analysis by Aird et al (371) (South Africa), 35 ART-naïve participants underwent various methods of internal and external fixation following an open fracture. The proportion of early wound infections in HIV-positive group was 15% (n=33), whereas the HIV-negative participants had a 22% (n=86) infection rate (risk ratio (RR) 0.69 (95% CI 0.3 to 1.7). It is important to note that Aird et al's results showed variation infection rates among the Gustilo-Anderson grades, with higher rates of infection in grade-I and -II, compared to III. Similar wound infection rates of 5% (n=39) were reported by Nawale et al (365) and other smaller studies have found similar findingss.(372, 373)

Conversely, Bates et al (368) (Malawi) reported the outcomes of 21 HIV-positive individuals in a prospective single blind cohort study who underwent a number of different forms of fracture fixation, including K-wire, screws, plates and IM nails. The infection rate was 33% in their study population, while the HIV-negative participants

had an infection rate of 15% (n=81). Only 5% (n=21) of this study population were on ART preoperatively, which increased to 16% postoperatively.

The majority of the smaller studies (< 20 patients) reported high rates of infection in HIV-positive individuals managed operatively following open an fracture.(18, 364, 365, 367, 374, 375) However, due to the small number of participants included in these studies, caution needs to be used when interpreting their results.

It is challenging to accurately draw valid conclusions from the studies discussed due to the deficiencies in study design, patient numbers and the reporting of outcomes. However, there is an apparent trend towards higher rates of infection in open fractures, irrespective of treatment method, although more research in this area is needed.

Table 6-4 Wound infection and open fractures (fracture management inclusive of internal fixation and external fixation)

Author	Year	Study type	Geographical location	Follow up period/months	Nº of patients on ART prior to study	Nº of patients on ART at follow-up	Definition of early infection	Outcome				HIV staging categories	Statistical analysis	Standard methods of evaluation
								Wound infection		Pin track infection				
								Study	Control	Study	Control			
G.D. Paiement (364)	1994	RSB	USA (San Francisco)	6.5	-	0/11 [0%]*	30 days	4/11 [36%]	10/118 [9%]	-	-	-	P=0.035, Chi-square.	CDC definition.
E.D. O'Brien (375)	1994	R	USA (New York)	Range 6-24	-	0/4 [0%]*	Range 6-24 months	4/4 [100%]	1/11 [9%]	-	-	-	-	-
W.J. Harrison (18)	2002	PSB	Africa (Malawi)	3	0/12 [0%]	0/12 [0%]	Three months	5/12 [42%]	3/27 [11%]	-	-	WHO staging 0=2 [80%], 1=3 [8%], 2=2 [5%], 3=2 [5%] Not known=1 [2%] CD4 counts >500=16%, 200-500=49%, <200=35%	P value = 0.084.	ASEPSIS wound score >10.

Author	Year	Study type	Geographical location	Follow up period/months	Nº of patients on ART prior to	Nº of patients on ART at	Definition of early infection	Wound infection		Pin Track Infection		HIV staging categories	Statistical analysis	Standard methods of evaluation
								Study	Control	Study	Control			
W.J. Harrison (20)	2004	PSB	Africa (Malawi)	6	0/7 [0%]	0/7 [0%]	Three months	-	-	5/7 [71%]	4/21 [19%]	WHO staging - 0=4 [57%], 1=1 [14%], 2=2 [29%] CD4 <200=2 [29%], 200-500=2 [29%], >500=[14%] NK 29%	P value =0.02.	Pin track – Checketts.
F.F. Birkholtz (372)	2005	P	Africa (South Africa)	N/M	0/16 [0%]*	0/16 [0%]*	N/M	0/3 [0%]	5/16 [31%]	-	-	N/M	N/M	N/M
A. Baburam (374)	2005	PSB	Africa (South Africa)	4	0/3[0 %]*	0/3 [0%]*	Four months	1/3 [33%]	0/2 [0%]	-	-	N/M	N/M	All fractures GA grade-II
A. Baburam (376)	2005	PSB	Africa (South Africa)	Mean 7.3 (rang e1-14)	0/10 [0%]	0/10 [0%]*	7.3 (range1-14)	1/10 [10%] GA grading I-0/1, II-0/3, IIIa-1/3, IIIb-0/3	1/8 [13%] GA grading II-0/5, IIIa-0/1, IIIb-1/2	-	-	N/M	P-value =0.641	N/M

Author	Year	Study type	Geographical location	Follow up period/months	Nº of patients on ART prior to	Nº of patients on ART at follow-up	Definition of early infection	Wound infection		Pin Track Infection		HIV staging categories	Statistical analysis	Standard methods of evaluation
								Study	Control	Study	Control			
S. Nawale (365)	2006	R	Asia (India)	N/M	0/14 [0%]	0/14 [0%]	N/M	8/14 [57%]	3/14 [21%]	-	-	CD4 counts were between 250-500. Values not mentioned.	N/M	ASEPSIS wound score.
S. Nawale (365)	2007	R	Asia (India)	12	0/39 [0%]	0/39 [0%]	One year	2/39 [5%]	-	-	-	N/M	N/M	N/M
A.R. Norrish (377)	2007	PSB	Africa (Malawi)	2	-	0/15 [0%]	Two months	-	-	9/15 [60%] Checketts II-7, III-1, IV-1	7/35 [20%] Checketts II-6, III-1	N/M	P-value =0.01	Checketts.
A. Abalo (367)	2010	R	Africa (Togo)	27	16/36 [44%]	16/36 [44%]	27 months	6/8 [75%]	-	-	-	CD4 count >500=21 [58%], 200-500=12 [33%], <200=3 [8%] Infection rates higher in symptomatic patients.	P value <0.05 sig P for CD4 counts >2=0.041	N/M

Author	Year	Study type	Geographical location	Follow up period/months	Nº of patients on ART prior to	Nº of patients on ART at follow-up	Definition of early infection	Wound infection		Pin Track Infection		HIV staging categories	Statistical analysis	Standard methods of evaluation
								Study	Control	Study	Control			
J. Aird (371)	2011	P	Africa (South Africa)	3	-	0/33 [0%]	30 days	5/33 [15%] GA grade-abrasions-0/5, I-4/14, II-1/9, IIIa-0/3, IIIb-0/2	19/86 [22%] GA grade-abrasion-4/14, I-3/32, II-4/17, IIIa-3/12, IIIb-4/11	-	-	CD4 count <350=15 [58%], <100=0, seven patients were not measured due to disease denial.	P value for infection= 0.49 in advanced HIV Risk ratio =1.46 [95% CI=0.6-3.7; 0.3-1.7, P value 0.4. In HIV-positive patients	ASEPSIS wound score.
J. Bates (368)	2012	PSB	Africa (Malawi)	1.5	7/139 [5%]	22/139 [16%]	Six weeks	7/21 [33%]	12/81 [15%]	-	-	CD4 count >500=27 [22%], 500-200=69 [56%], >500=28 [23%] ASEPSIS/CD4 no relationship	Open HIV-positive versus HIV-negative p =0.064 not significant.	ASEPSIS wound score >10.

Author	Year	Study type	Geographical location	Follow up period/months	Nº of patients on ART prior to	Nº of patients on ART at follow-up	Definition of early infection	Wound infection		Pin Track Infection		HIV staging categories	Statistical analysis	Standard methods of evaluation
								Study	Control	Study	Control			
N.E. Howard (370)	2013	P	Africa (South Africa)	1-3	3/28 [11%] GA gradi ng I-1, IIIa-1, IIIb-1	3/28 [11%] GA grading I-1, IIIa-1, IIIb-1	30 days	3/28 [11%] GA grading II-2/11	11/57 [20%] GA grading II-3/40, III-7/17	3/17 [18%] Only those with Checketts score >4	5/40 [13%] Only those with Checketts score >4	Mean CD4 = 432 (104-1190) No relationship between CD4 and ASEPSIS score. 4 had a CD4<350	P value for wound infection in HIV-positive p=0.32, RR=0 55, 95% CI=0.17-1.8; P value for pin track=0.4 7, RR 1.62,95% CI 0.44 to 6.07	ASEPSIS wound score >10, Checketts score of 4 for pin track infection.
N. Ferriera (378)	2014	R	Africa (South Africa)	5	0/40 [0%]	25/40 [63%]	23 weeks (range 6-104)	-	-	8/40 [20%] Checketts - II-6, III-1, IV-1	36/168 [21%] Checketts- II-26, III-6, IV-2, VI-2	Mean CD4=347.4, SD+/-162.4, rang =82-682. No relationship between CD4 and infection.	P=0.9	Checketts score.

Key: P - prospective study; PSB - prospective single blind study; R - retrospective study; RSB - retrospective single blind study, KN – Not known, N/M – not mentioned

6.4.1.2.2 Pin track infection

Four studies focused on analysing the proportion of pin track infections in HIV-positive patients managed with external fixators following open fractures (Table 6-4). All of the studies classified pin track infections using the Checketts (379) scoring system. Howard et al (370) (South Africa) reported outcomes of 17 HIV-positive participants and showed severe (grade-V or -VI) pin track infection rates of 18%, whereas the infection rate for the HIV-negative control group 13% (n=40). In the retrospective study by Ferreira et al (380) (South Africa) pin track infections of a Checketts score ≥II were reported. The proportion of pin track infections in HIV-positive participants was 20% (n=40), 63% of which were commenced on ART postoperatively. A similar pin track infection rate of 21% (n=168) was reported in the HIV-negative study cohort. Ferreira et al (380), included a third group of participants of unknown HIV status and they had pin track infection rate of 24%. There was no statistically significant difference for incidence or severity between the three groups reported. (380)

Norrish et al (377) (Malawi) reported results from 15 HIV-positive participants stabilised with an external fixation, who were not taking ART. This study population had a pin track infection rate (Checketts score>II) of 60% compared to 20% in the HIV-negative participants (n=35). Only one study participant required surgical intervention for their infection. Harrison et al (20) (Malawi) demonstrated a 75% rates of pin track infection in seven cases, while 21 controls in their study had infection rates of 19%.

Mixed rates for pin site infection have been shown in HIV-positive individuals undergoing external fixation for fracture fixation, with all but one research group demonstrating higher rates of infection in HIV-positive patients. Limitation again include the study designs and number of participants included in the studies. Additionally, the care of pin tracks has been changed in many centres worldwide in recent years with reported improvements in outcomes.(381) The current published

literature does not include these newer treatment regimens. In conclusion, from the evidence available, the proportion of pin site infections appears to be higher in HIV-positive patients, but more research is required.

6.4.2 Long-term outcomes

6.4.2.1 Late implant infection

There were five studies that investigated late implant infection in closed and open fractures (Table 6-5). Late implant infection was defined as deep infection, which became evident six or more months after fracture surgery.

In prospective studies, Harrison et al (382) (Malawi) (n=26) and Graham et al(383) (Malawi) (n=103) reported that there were no late implant infections in HIV-positive trauma patients who sustained closed fractures that underwent fracture fixation surgery. The mean follow-up in these two studies was 12 and 27 months respectively. None of the study population were taking ART in the Harrison study pre-operatively but 8% had started ART post-operatively. In the study population in the study reported by Graham et al(383)(Malawi) 27% of postoperative HIV-positive participants were taking ART at follow-up.

In a prospective study undertaken by Keetse et al (384) (South Africa), 12-month late implant infection rates were 3% for both HIV-negative (n=120) and HIV-positive (n=40) study groups. Brijlall (385) (South Africa) reported 18 of 21 late implant infections were seropositive in HIV-positive individuals and patients presented a mean of 24 months (no range given) after fracture surgery. Neither of the studies reported the definition of late implant infection, which is likely to have had a major bearing on the recorded rates of infection.

In terms of late infection following an open fractures, Graham et al (383) (Malawi) did not find any cases in twelve study participants. Phaff et al (386) (South Africa) deonstrated a late implant infection rate of 8% in both the HIV-positive study population, and the HIV-negative cohort.

In summary, HIV does not appear to be a risk factor for late implant infection following fracture fixation for closed injuries. Two small study reported similar results in patients with open injuries.

Table 6-5. Late implant sepsis in open and closed injuries

Author	Year	Study type	Geographical location	Follow-up period (months)	ART prior to study % of patients	ART (%) at follow-up	Outcome				HIV staging categories	Statistical analysis	Limitations
							Late implant sepsis						
							Closed		Open				
							Study	Control	Study	Control			
S. Brijlall (387)	2003	R	Africa (South Africa)	-	0/18 [0%]*	0/18 [0%]*	18◇	-	-	-	N/M	N/M	No control. No blinding. Follow up was not defined.
W.J Harrison (20)	2004	P	Africa (Malawi)	12	0/26 [0%]*	0/26 [0%]*	0/26 [0%]	-	-	-	CD4 >500=7 [27%], 200-500=8 [31%], <200=9 [35%]	N/M	No control. No blinding No standard method of scoring outcomes such as ASEPSIS.
M. Phaff (386)	2015	P	Africa (South Africa)	39 (mean)	0/13 [0%]	1/13 [0%]	-	-	1/13 [8%] GA grading abrasions 0/3, I-0/6, II- 0/3, IIIa- 0/0, IIIb=1/1	2/24 [8%] GA grading abrasions 0/5, I=1/11, II=0/3, IIIa- 0/3, IIIb=1/2	CD4>50=2 [15%] 200-500=4 [31%] <200=2 [15%] Unknown=5 [39%]	RR in open fractures= 0.92 (CI 0.092-9.2) P value =1 (Fisher exact test)	No control. Small sample size. Not blinded. No standard method of measuring outcome. High rate of loss to follow-up.

M.M. Keetse (384)	2014	P	Africa (South Africa)	12	12/40 [30%]	12/40 [30%]	1/40 [3%]	3/120 [3%]	-	-	CD4<35= in seven patients	N/M	Not blinded. Abstract.
S. Graham (388)	2015	PSB	Africa (Malawi)	27 (mean)	7/103 [6%]	25/103 [24%]	0/93 [0%]	-	0/12 [0%]	-	CD4 > 500= 21 [23%] 200-500=58 [62%], <200=14[15 %]	N/M	No control, Treatment was not blinded/randomised beyond initial early postoperative period
Key: P - prospective study; PSB - prospective single blind study; R - retrospective study, RR – relative risk N/M- not mentioned													

6.4.2.2 Non-union

In all of the non-union studies, a clear definition of the method for determining fracture union, either radiologically or clinically, was not reported, making accurate interpretation of the results challenging. There were sustainial difference in duration of follow-up, showing the lack of consistency of these studies. For this review, a delayed union was defined as a fracture that was not healed at six months and a non-union was defined as a fracture that had not healed at 9 months. Clinically, union was considered to be present if there was return of function and weight bearing and a pain-free range of motion, whereas for radiographic union three out of the four cortices on anterior-posterior and lateral X-rays had to be bridged.(123)

6.4.2.2.1 Non-union in closed fractures

Eight authors reported non-union in closed fractures, with a mix of prospective(382, 384, 389) and retrospective studies. (367, 390) (Table 6-6)

Harrison et al (Malawi) showed a 0% non-union rate in 26 HIV-positive ART-naïve participant that were clinically and radiologically assessed.(382) Gardner et al (Malawi) reported a 5% non-union rate in 95 HIV-positive individuals in a similar setting (389), 6% of whom were taking ART.

Keetse et al (384) (South Africa) investigated 40 closed femoral fractures in HIV-positive patients that had undergone IM nailing for fracture fixation. However, the method of evaluation of non-union was not reported. One participant in the HIV-positive participants was on ART at the time of follow-up. None of the 40 study participants developed non-union, whereas the HIV-negative control group (n=120) had a rate of non-union of 2%.

Abalo et al (367) (Togo) did not describe how they defined non-union, and showed rates of 11% (n=4) non-union in their HIV-positive group of 36 participants. Cummins et al (390) (Republic of Ireland), in a retrospective study, also failed to give a definition of non-union, and reported three of the four HIV-positive patients developed a non-union. Hao et al (369) (USA) reported outcomes from a cohort of 24 HIV-positive patients, 92% of which were taking ART, with closed fractures, none of whom developed non-union.

Babruam (374) (South Africa) followed up 11 closed fractures in their study population of ART-naïve participants that underwent IM nailing fixation and showed that all had united within four months of surgery. Brijlall (385) (South Africa) examined an ART-naïve HIV-positive cohort of 18 participants with infected implants postoperatively and reported a rate of non-union of 11%. Neither Babruam (374) nor Brijlall (385) presented the method of evaluation of non-union.

All the eight studies reviewed were poorly designed, with no consistent definition of union and none used a validated radiological scoring system, making it difficult to draw any valid conclusion from the current clinical literature available.

6.4.2.2.2 Non-union in open fractures

Six study groups investigated non-union in open fractures. Aird et al (391) (South Africa) reported rates of non-union of 15% (n=33) in HIV-positive patients and 4% (n=100) in the HIV-negative controls. However, the method of evaluation, length of follow-up, grade of open fracture and energy of the initial injury were not recorded.

In a prospective analysis of 13 HIV-positive individuals, Phaff et al (386) (South Africa) reported a rate of non-union of 8% (n=1). There was one patient who was on ART at the time of study follow-up, who did not develop a non-union. The proportion of non-unions in the HIV-negative control group was 0% (n=24). Union was assessed both

clinically and radiographically in this study. However, due to the low numbers, it is difficult to draw valid conclusions from this study.

A retrospective study by Nawale (392) (India) of 39 patients, not on taking ART, demonstrated rates of non-union of 10%. In five study participants Gardner et al (389) (Malawi) described a non-union rate of 20%. These two authors assessed patients for non-union on radiological imaging.

Prospective studies undertaken by Harrison et al (20) (Malawi) and Babruam (374) (South Africa) followed patients up for less than 9 months, therefore not fulfilling this thesis's criterion of non-union. Harrison et al (20) followed their study population up for six months using clinical evaluations and radiographs. They reported a 43% (n=3) rate of delayed union in seven patients. Babruam (374) followed three patients who were HIV-positive for four months and reported that one patient (33%) did not show fracture union at the end of follow-up, whereas the two HIV-negative patients had full fracture union. These two studies were the only ones to report any outcomes in related to delayed fracture union. However, due to the low number of study participants included, no conclusions can be draw from this data.

Similar deficiencies identified when reporting infection outcomes in HIV positive individuals are evident in studies undertaken investigating delayed and non-union in closed fractures. Studies include small patient numbers, there is a lack of a clear statistical plan and a prospective power calculation and poor overall study design. However, for open injuries, despite these deficiencies, evidence suggests that HIV-positive patients who have an open fracture may be at higher risk of developing delayed and/or non-union compared to HIV-negative patients. However, again more research is needed to give a definitive answer.

Table 6-6. Non-union in open and closed fractures

Author	Year	Study type	Geographical location	Follow-up period/months	ART prior to study % of patients	ART (%) only in study	Outcome				Categories	Statistical analysis	Standard methods of evaluation
							Non-Union						
							Closed		Open				
							Study	Control	Study	Control			
S. Brijlall (385)	2003	R	Africa (South Africa)	N/M	0/18 [0%]*	0/18 [0%]*	2/18 [11%]	-	-	-	N/M	N/M	Radiological assessment.
W.J. Harrison (20)	2004	PSB	Africa (Malawi)	6	0/7 [0%]	0/7 [0%]	-	-	3/7 [43%]	1/21 [5%]	WHO staging; 0=4 [57%], 1=1 [14%], 2=2 [29%], CD4 count; > 500=2 [29%], 200-500= [29%], >5001 [14%] unknown 2 [29%]	Infection: P value =0.02 Union: p-value =0.059.	Clinical and radiological assessment.
W.J. Harrison (382)	2004	P	Africa (Malawi)	12	0/26 [0%]*	0/26 [0%]*	0/26 [0%]	-	-	-	CD4 >500=7 [27%], 200-500=8 [31%], <200=9[35%]	-	Clinical and radiological assessment.

Author	Year	Study type	Geographical location	Follow-up period/months	ART prior to study % of patients	ART (%) only in study	Outcome				Categories	Statistical analysis	Standard methods of evaluation
							Non-Union						
							Closed		Open				
							Study	Control	Study	Control			
A. Baburam (393)	2005	PSB	Africa (South Africa)	4	0/14 [0%]*	0/14 [0%]*	0/11 [0%]	0/14 [0%]	1/3 [33%]	0/2 [0%]	N/M	N/M	N/M
S. Nawale (392)	2007	R	Asia (India)	12	0/39 [0%]*	0/39 [0%]*	-	-	4/39 [10%]	-	N/M	N/M	Clinical and radiological assessment.
A. Abalo (367)	2010	R	Africa (Togo)	27	16/36 [44%]	16/36 [44%]	4/36 [11%]	-	-	-	CD4>500=21 [58%], 200-500=12 [33%], <200=3 [8%] Infection rates higher in symptomatic.	P value <0.05	-

Author	Year	Study type	Geographical location	Follow-up period/months	ART prior to study % of patients	ART (%) only in study	Outcome				Categories	Statistical analysis	Standard methods of evaluation
							Non-Union						
							Closed		Open				
							Study	Control	Study	Control			
R.O.E. Gardner (389)	2012	P	Africa (Malawi)	12	5/96 [5%]	6/95 [6%]	5/95 [5%]	-	1/5 [20%]	-	CD4 >500= [21%], 200-500= [64%], >500= [21%] no relation between CD4 and non-union.	N/M	Clinical and radiological assessment.
J. Aird (391)	2012	P	Africa (South Africa)	N/M	0/33 [0%]*	0/33 [0%]*	-	-	5/33 [15%]	4/100 [4%]	N/M	P=0.04 for non-union, risk ratio=4.	N/M
F. Cummins (394)	2014	R	Europe (Republic of Ireland)	25	12/17 [71%]	13/17 [76%	3/4 [75%]	-		-	CD4 > 500=5, 200-500=12, >500=0	Correlation coefficient for complications and ART use=-0.35 determination = 0.12. Non-significant.	N/M

Author	Year	Study type	Geographical location	Follow-up period/months	ART prior to study % of patients	ART (%) only in study	Outcome				Categories	Statistical analysis	Standard methods of evaluation
							Non-Union						
							Closed		Open				
							Study	Control	Study	Control			
M.M. Keetse (384)	2014	P	Africa (South Africa)	12	12/40[3%]	12/40[3%]	0/40 [0%]	2/120[2%]	-	-	Seven patients with CD4<350	N/M	N/M
M. Phaff (386)	2015	P	Africa (South Africa)	39 (mean)			-	-	1/13 [8%] GA grading abrasions=0/3, I=0/6, II=0/3 IIIa=0/0 IIIb= 1/a	0/24 [0%] GA grading abrasions=0/5, I=0/11, II=0/3, IIIa=0/3, IIIb=0/2	CD4>500=2 [15%] 200-500=4 [31%] <200=2 [15%] Unknown=5 [39%]	N/M	Clinical and radiological assessment.
J. Hao (369)	2015	P	USA (Denver)	12	22/24 [92%]	22/24 [92%]	1/24 [4.2%]	-	-	-	CD4>500=6/21, 200-500=10/21, <200=5/21	N/M	Clinical and radiological assessment.

Key: P - prospective study; PSB - prospective single blind study; R - retrospective study

N/M - not mentioned

6.5 Summary

There have been a number of studies with appropriate length of follow-up and number of patients that have investigated early infection in closed fractures, demonstrating no increased risk of infection in HIV-positive patients. (365), (366), (368)

In the studies reporting pin track infections, there were varied rates of infection using a consistent scoring method, in the Checketts scoring system. All but one research group reported higher proportions of infection in HIV-positive individuals. A number of limitations surround the study designs and patient numbers included. However, from the evidence available, the rate of pin site infection seems to be higher in HIV-positive patients.

Mixed results have been demonstrated for early wound infection rates in HIV patients with open fractures. Aird et al documented the largest study with lower rates of wound infection in the HIV-positive patients compared to HIV-negative controls but had significant variations among the Gustilo-Anderson grades of open fractures. Participants with a grade-I Gustilo-Anderson had a delay in their time to debridement in this study, which could potentially explain the higher than expected rates of infection observed in the grade-I open fractures. Excluding Howard's study, all the other studies reported an increased risk of infection in the HIV-positive patients following an open fracture. The differences in the quality of these studies reported so far makes it difficult to draw clear conclusions, and more well-designed and standardised studies including Gustilo-Anderson grading and other key paramters already discussed are needed.

Low rates of late implant infection in the HIV-positive patients with closed fractures have been demonstrated in studies so far. In open fractures, there is minimal data available to draw any valid conclusions.

In all of the studies evaluated in this systematic review, there was a lack of a clear definition of delayed and non-union to allow consistent evaluation between the studies. Fracture union is dependent on a huge number of different variables, many of which have been discussed in previous chapters.(395, 396) All the studies included were poorly designed, including no definition of union and none used a validated radiological scoring system for bone union, such as the RUST Score.(123, 124) There was also a lack of controls to compare fracture healing outcomes, small patient numbers were reported, and no study demonstrated a clear prospective power calculation or statistical plan to definitively give an answer to the question of whether HIV increases your risk of delayed or non-union following a fracture. Therefore, it is difficult to draw any valid conclusions from the studies reviewed in this systematic review. As discussed in the previous chapter, pre-clinical science research has suggested that HIV infection may be associated with delayed and non-union of fractures.(19) However, the lack of robust clinical evidence highlights the importance of determining the effect of HIV on fracture healing.

No study included enough participants taking ART to draw meaningful conclusions about the effect of ART on the fracture repair process. There has been a potential association between issues of impaired fracture healing and the use of ARTs.(19) However, the multiple sources of heterogeneity such as duration of treatment, different drug regimens and differing degrees of immunosuppression make it difficult to assess the any effect. ART might also be expected to improve wound infection rates after open fractures and to reduce late implant infection in HIV-positive individuals, but current data lacks power to enable any such conclusions to be drawn.

6.5.1 Systematic review limitations

The systemic review inclusion criteria ensured that all available literature was analysed, including abstracts that were not published as full papers, resulting in some loss of detail relating to study design and definitions. Other limitations were that any articles that were not in the English language were excluded. However, the majority of researchers publish in English and this is unlikely to have missed any major papers.

6.5.2 Conclusions of systematic review

Historically, there have been suggestions that fracture surgery on HIV-positive individuals should be avoided and, if undertaken at all, removing the implants following fracture union should be considered to reduce the risk of infection.(397) This systematic review confirms that from current available clinical data, the surgical management of fractures in the HIV-positive groups is not contra-indicated.

The outlook in the closed fractures is very encouraging, as it appears comparable to infection rates obtained in HIV-negative patients with equivalent injuries. The effect of ART on bone healing is uncertain and has not been sufficiently investigated.

There are a number of areas where more research is necessary; in particular, the effect of HIV and ART on wound infection rate after open fractures, as well as the impact of HIV and ART on fracture healing. The focus of this thesis will aim to give a clearer answer to the latter problem, if HIV and its treatment are a risk factors for the development of delayed union or non-union following fracture surgery.

CHAPTER 7. GENERAL METHODOLOGY

7.1 Aims of this chapter

This chapter discusses methodological aspects that are common to the HOST 1 and HOST 2 studies, including study sites, laboratory procedures, data management and ethical considerations. Methods pertaining to each study – namely study design, population, clinical definitions, procedures, sample size, statistical analysis and timescale, will be discussed under the individual chapters.

7.2 Study sites

7.2.1 Groote Schuur Hospital

Groote Schuur Hospital (GSH) is the main teaching hospital of the University of Cape Town's Medical School (Figure 7.1). The name Groote Schuur is Dutch for 'Great Barn' and the hospital was named after the original Groote Schuur estate built in the 17th century by Dutch settlers when the city of Cape Town was founded. The original hospital building was built with the aid of public subscription and government funding. In 1932, building started and the hospital was completed and opened in 1937. Constructing the new hospital commenced in 1983 and it opened in 1989. GSH is world famous for being the institution where the first ever human-to-human heart transplant took place, performed by the surgeon Christiaan Barnard.

The orthopaedic trauma department is a Level 3 trauma unit and has a total of 92 acute orthopaedic admission beds, providing a predominantly adult-orientated service. There are eight full-time orthopaedic consultants and 24 registrars working within the department. In 2017, there were approximately 1850 orthopaedic admissions and 1704 orthopaedic trauma surgeries performed. (Personal communication Professor Sithomo Maqungo)

GSH and TBH provide a split tertiary service to the Western Cape, covering a population of approximately six million people. However, the Northern and Eastern Cape do not have a functional tertiary hospital, therefore this creates a combined estimate of an extra 14.2 million people who would see GSH or TBH as their nearest tertiary referral trauma hospital.(Figure 7-1)

Figure 7-1. Groote Schuur Hospital – the new hospital in the left front foreground and the old hospital in the back right.

7.2.2 Tygerberg Hospital

Tygerberg Hospital (TBH) is a tertiary hospital located in Belville, Cape Town, South Africa, 20 km from GSH (Figure 7-2, Figure 7-3). The hospital was officially opened in 1976 and is the largest hospital in the Western Cape, with over 1900 hospital beds, making it the second-largest hospital in South Africa. It acts as a teaching hospital in conjunction with Stellenbosch University.

The Division of Orthopaedic Surgery of Tygerberg Academic Hospital is also a Level 3 trauma unit and has a total of 160 acute admission beds for trauma and emergencies, providing an adult and paediatric service. The department has 11 orthopaedic

consultant surgeons, 18 junior registrars and approximately 600 orthopaedic trauma admissions per month.

Figure 7-2. Tygerberg Hospital – the university is the building at the front and the hospital is located at the back.

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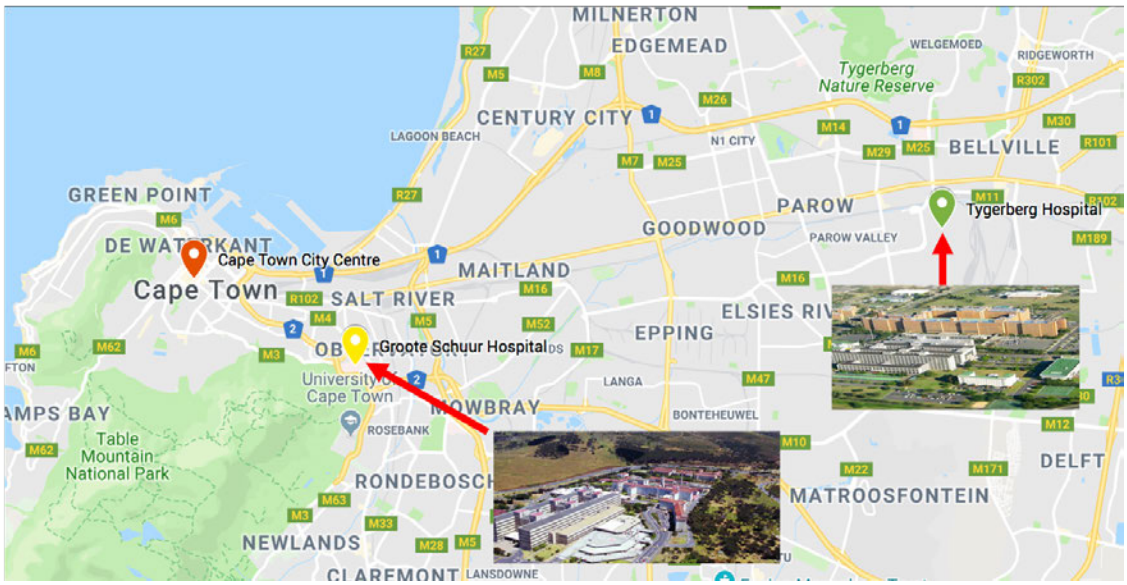
Figure 7-3. Locations of Cape Town city centre (red), Groote Schuur (yellow) and Tygerberg Hospitals (green).



7.2.3 The Cape Flats

The Cape Flats is the name given to an area of land situated to the southeast of the central city region of Cape Town.(Figure 7-4) From the 1950s onwards, the area became home to people that the South African apartheid government designated as non-white. The government forced non-white people out of more central urban areas designated for white people and into government-built townships in and around the Cape Flats. The Cape Flats is now home to much of the population of Greater Cape Town. (398) It is also where the poorest communities are located and many of the houses are non-permanent settlements, where crime, violence and unemployment are high. Furthermore, it's in these areas where HIV is most prevalent.(398) It is also the main drainage area for both of the study site hospitals.

Figure 7-4. An area map of the approximate borders of the Cape Flats. (398)

7.3 Staff

The following staff were employed to conduct the study:

- Two research nurse coordinators (Nomsa Yekiso and Nosipho Mncwabe): responsible for consent and enrolment of participants during recruitment period; taking the majority of clinical specimens; coordinating participant follow-up and field worker visit schedule; undertaking telephone follow-up of study participants: HIV and ART counselling and HIV clinic referral and follow-up.
- One database designer: responsible for the design of the Redcap database and initial database testing.
- One database manager: responsible for maintenance of the database and raising any queries related to data collection.

In addition to screening and enrolment of study participants, study staff also contributed to routine clinical activities at both GSH and TBH.

7.4 Laboratory procedures

7.4.1 Sample registration and storage

All clinical specimens collected from study participants were processed at the GSH or TBH on-site laboratories. Both laboratories participate in the UK National External Quality Assessment Scheme (NEQAS).

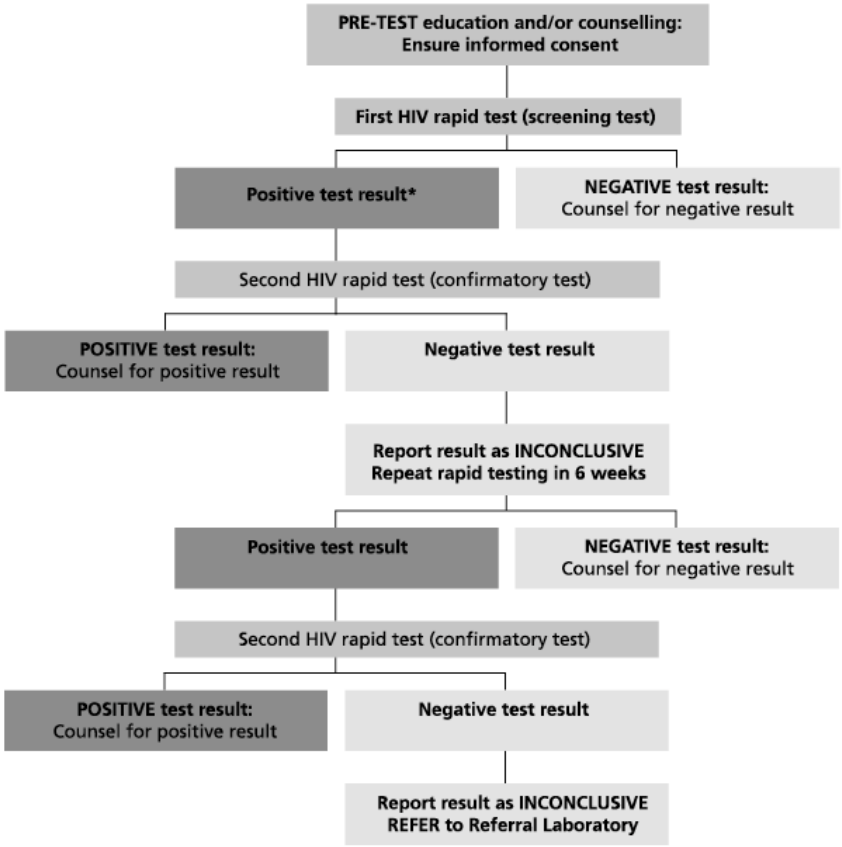
Each study specimen was labelled with a pre-printed hospital identification number and person details, with a corresponding laboratory specimen submission form that contained the same details, in addition to the participant's name and date of birth. This allowed treating clinicians to access to the results from the National Health Laboratory Service (NHLS) in a timely fashion. Laboratory test results were subsequently linked to the participants' demographic information by their unique hospital number.

7.4.2 HIV test, CD4 count and viral load measurement

The HIV status of study participants was determined using rapid HIV tests as per WHO-recommended algorithm and national guidelines (Figure 7-5) (50)); Alere DetermineTM HIV-1/2 assay (Alere Medical Co. Ltd., Chiba, Japan) was used as the initial screening test, and Uni-GoldTM Recombigen HIV test (Trinity BioTech, Wicklow, Ireland) as the confirmatory test.

CD4+ T-cell counts were performed on a FACScount™ flow cytometer (Becton Dickinson, BD Biosciences, San Jose, USA). The viral load measurement was performed using the bioMe'rieux NucliSENS EasyQ System HIV-1 QT test.

Figure 7-5. Algorithm for use of the rapid HIV test (50)



7.5 Data management

Standard operating procedures for data management without breach of confidentiality were followed. The HOST study utilised two custom-made 'REDCap' databases hosted at the University of Cape Town, South Africa. REDCap (Research Electronic Data Capture, Vanderbilt University, Tennessee, USA) is a secure, web-based application designed to support data capture for research studies. The system provides audit trails for tracking data manipulation. Each study subject was allocated a unique identifier. Data on subject demographics, baseline history of injury, treatment and surgery were recorded, as well as risk factors for delayed bone healing and/or non-union. This information was recorded manually by the HOST study team, onto specially designed, paper-based data collection forms. Data from the paper forms was entered onto the study 'REDCap' database by Nomsa Yekiso (NY) and

Nosipho Mncwabe (NM) (study research nurse, Figure 7-6). Once the data was entered, a series of steps was taken to check for transcription errors, including the nurses checking the data of each other's entry for all patients. Data entry for each data form was checked by me and finally by the Clinical Research Centre database management team at the University of Cape Town, with 100% quality assurance on critical variables.

Figure 7-6. Research nurses Nomsa Yekiso (left) and Nosipho Mncwabe (right).

Once the dataset for a particular analysis was complete, a manual (visual) and automated (the R for Statistical Computing) checking of critical variables was performed. Outliers thus identified (defined as a value that is more than three standard deviations from the mean) were then checked manually against original data forms. A number of minor errors were found and corrected before analysis.

Laboratory results were received in a web-based format via the NHLS Prelink National Laboratory Information Management System. The research nurses entered these results into the study database and subsequently went through the same checks described above. The database was stored on centrally managed servers that were

protected against data loss. Data was extracted from the database for analysis into either Microsoft Excel (Microsoft, Seattle, Washington USA) or the R Project for Statistical Computing version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Patients' BMD was measured using a DEXA Heel Scanner, Calscan DXL (Rothband Medical Imaging Supplier). This information was stored on a Calscan DXL Application, located on an assigned password-protected study laptop computer. These results were then entered onto the database using the methods described above.

7.6 Statistical analysis

Statistical analysis plans are described in the Methods section of the individual studies. All analyses were undertaken using 'R' for Mac OS statistical computing software (R 3.6.1 GUI 1.70 El Capitan build [7684]). Missing observations were included in the analysis of each study by creating missing value categories.

7.7 Ethical approval

The HOST 1 and 2 study received local and the University of Cape Town and University of Stellenbosch Faculty of Health Science Human Ethics Committee approval in July 2017 – reference number 590/2016 and N17/05/052. Furthermore, approval from Liverpool School of Tropical Medicine Research Ethics Committee (protocol number: 17.013) was gained.

The principal investigator and collaborators declared that this submission was in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the Second World Conference on Research Integrity in Singapore, 2010.

There were several subsequent amendments to address minor study modifications. See Appendices (Appendix 13-2, Appendix 13-3) for details of the consent forms and patient information sheets in English (Xhosa, Afrikaans and Zulu translations were also used but are not included in this thesis). Written informed consent was obtained from all subjects and the say consent and patient information was used for both studies.

HIV testing was offered to all patients of our study units, based on the recommendations of the Department of Health HIV Counselling and Testing Policy Guidelines. Since a substantial proportion of participants were HIV-positive, every effort was made to safeguard patient confidentiality.

All study team members underwent Good Clinical Practice training prior to commencement of the study. Informed consent was obtained from all study participants, either written or witnessed verbal consent, with thumbprint if the participant was illiterate.

CHAPTER 8. HIV IN ORTHOPAEDIC SKELETAL TRAUMA STUDY 1: Aims, Design, Methodology and a Descriptive Analysis of the Study Cohort

8.1 Aims of this chapter

In this chapter a description of the study aims, methods and a descriptive analysis of the HIV in Orthopaedic Skeletal Trauma (HOST) 1 study cohort will be presented.

8.2 Introduction

There have been no previous large, appropriately powered, prospective studies undertaken investigating the effect of HIV on fracture healing. As already discussed, there have been studies undertaken in the past, but most have been retrospective. In all of the studies evaluated to date, there was a lack of a clear definition of delayed or non-union to allow consistent evaluation between the studies. None of the studies used a validated radiological scoring system for bone union, such as the RUST score. The largest study by Gardner et al (389) investigated bone union in an extremely heterogenous cohort of HIV-positive participants, who had numerous types of fractures and had undergone several different forms of fracture fixation. The primary author of this thesis was also involved in the follow-up of this cohort of participants, assessing the incidence of late infection, and published these results.(388) The above findings and my previous research in Malawi (388), (399), (400) enabled a gap to be identified in the current literature and prompted this research.

The main hypothesis underlying this research is that HIV infection causes delayed bone union and is a risk factor for the development of non-union following a fracture. If this hypothesis were shown to be true, the surgical management of fractures could be tailored to optimise bone union during the fracture-healing phase in HIV-positive participants, improving outcomes and reducing the substantial physical and social burden that occurs in these participants as a result of traumatic injuries.(18) Furthermore, it may indicate the importance of future research to look at approaches

to minimise this risk, since the burden of trauma is an increasing problem in low and middle-income countries where people with HIV infection are living longer.(213)

8.3 Aims and objectives

The aim of the HOST 1 study was to establish whether HIV infection was a risk factor for the development of delayed bone union or non-union following a fracture.
The main objectives of the HOST 1 study were to:

1. Establish whether HIV infection is a risk factor for the development of delayed bone union or non-union following a fracture.
2. Assess other risk factors associated with delayed bone union or non-union in HIV-positive and -negative adults.
3. Determine the incidence of superficial, deep and late wound infections in HIV-positive and -negative participants following fracture surgery.

8.4 Methodology

8.4.1 Study design

A multi-centre prospective case-cohort study of participants undergoing fracture surgery within the Orthopaedic and Trauma Department at GSH and TBH, Cape Town, South Africa was undertaken. The study was undertaken over a 14-month period, between September 2017 until December 2018. Participants who underwent surgery had a HIV test and were subsequently followed up over a 12-month period to determine the proportion of participants whose fracture healed and those that developed delayed union and non-union.

The protocol for this study was published in the South African Orthopaedic Journal, 2018 and is available as an open access article, downloadable directly from the journal website on the following link - http://journal.saoa.org.za/index.php/saoj/article/view/250/243. (401) For this

publication, the author of this thesis (Simon Graham) established the concept, lead the write up, edits and final submission of the paper.

8.4.2 Study site

Participants were enrolled from the orthopaedic department of two tertiary referral trauma hospitals, GSH and TBH, in Cape Town, South Africa.

8.4.3 Study population

All participants older than 18 years of age with fresh (within two weeks of injury), closed and open, tibia and femur fractures who underwent intra medullary (IM) nailing for fracture fixation were potentially eligible for inclusion in the study. The following eligibility criteria were applied:

Inclusion criteria - Participants were eligible for this study if they:

- were 18 years or older at assessment
- had surgery undertaken at GSH or TBH
- underwent surgery within two weeks of injury
- were assessed for inclusion by the study team within two weeks surgery
- sustained a closed or open fracture of the tibia or femur
- underwent IM nailing for fracture fixation

Exclusion criteria - Participants were excluded from participation in this study if they had:

- pathological fracture
    - Fractures which occur from low energy injuries which occur through an area of bone weakness with a pre-existing abnormality
- intertrochanteric femur fractures
- paraplegia
- pre-surgical infection at the fracture site

- open injury for >48 hours before the first debridement
- severe burns
- evidence that the participant would be unable to adhere to study procedures, complete questionnaires or attend follow-up major head injury

Those who sustained multiple injuries had their injuries documented and were included in the enrolment process.

8.4.4 Study procedures

8.4.4.1 Enrolment

Participants who had undergone IM nailing for fracture fixation were approached post-operatively for consideration for inclusion in the study as stipulated by the ethics committee. Persons that met the eligibility criteria who were willing to consider participation in the study received a participant information document (translated into Xhosa, Zulu, Afrikaans or English). Written informed consent was obtained from the participant.

Study participants underwent a baseline questionnaire, and established risk factors for delayed bone healing and non-union were recorded.

The participants' full blood count, renal function, vitamin D level and albumin were assessed (Table 8-1). Due to the fact that participants were recruited following their operative procedure, the pre-operative haemoglobin was extracted from the laboratory records prior to surgery. All participants underwent HIV testing and a measurement of the participants' CD4 count and viral load if positive. If the participant was already known to be HIV-positive (previous laboratory confirmation required) and taking ART, their ART regimen was recorded, if appropriate. If a participant was found to have a new diagnosis of HIV, standard local hospital protocol was followed. This included the research nurses providing post-test counselling and

subsequent referral onto the local HIV treatment service. The study team subsequently kept in close contact with the participant during the weeks after the diagnosis and organised an earlier follow-up review if required.

8.4.4.2 Laboratory investigations

The investigations that were performed on enrolment are listed in Table 8-1. Note that throughout this thesis when referring to the measurement or value of vitamin D, this is reference to 25-hydroxyvitamin(25[OH]D).

Table 8-1.Participant enrolment laboratory investigations

Pre-operative	Post-operative
Haemoglobin	Haemoglobin Platelets White blood cells Creatinine Urea Albumin Vitamin D (25-hydroxyvitamin(25[OH]D) HIV test CD4 cell count HIV-1 RNA viral load

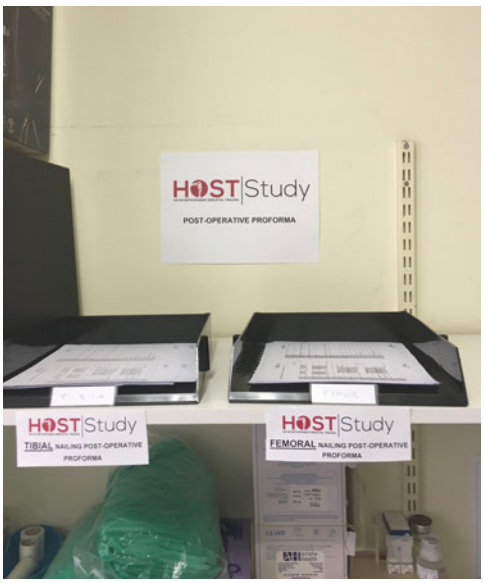
8.4.4.3 Screening and tracing

Screening and tracing were undertaken systematically. All participants who underwent IM nailing at both study sites had a surgical proforma completed by the operating surgeon. The operating surgeon documented the Gustilo Anderson grade on the study proforma following first wound debridement. If the participant had previously had a debridement for an open fracture, the operating surgeon determined the GA grade with input from the surgeon who undertook the initial

debridement. On all occasions the surgeon who undertook the first debridement for an open fracture was consulted before a score was assigned.

A picture of the proforma including the participant's name and base ward was uploaded to an encrypted smartphone application (WhatsApp) that all those involved in the study had access to. This proforma was then placed in the assigned area in the operating theatre for collection by the research nurses. (Figure 8-1) The research nurses subsequently collected the proforma and then reviewed the participants on the ward post-operatively to screen for enrolment. Furthermore, all participants waiting for and who had undergone IM nailing were added to a list during the morning trauma meeting and cross-referenced with the proformas collected to ensure no participants were missed. The research nurses also undertook daily screenings of inpatient admissions and discharges at both hospitals to ensure all possible candidates were screened for inclusion.

Figure 8-1. Assigned area in the operating theatre for placement of the completing surgical proformas for collection by the research nurses



To identify any participants missed using the above process, all trauma clinic attenders were screened on a daily basis by the study team to identify participants who were not considered for enrolment during their inpatient stay. All enrolled

participants in the study were reviewed as outpatients on one set day at both GSH (Wednesday) and TBH (Thursday). It was department policy for all participants who underwent IM nailing of the femur or tibia to be followed up in these two trauma clinics, irrespective of study enrolment. If any patient who had undergone IM nailing presented to the trauma clinic on any other day, the surgeon reviewing them organised a review by the research team that day to screen the participant for enrolment. This rarely happened, since the vast majority of participants were screened in hospital.

Following screening by the research nurses the participants were either enrolled or excluded from the study. If there were any concerns regarding eligibility for the study, the principal investigator (Simon Graham) personally reviewed the participant with the research nurses. Furthermore, if a participant declined enrolment, the research team reviewed them again during their admission to see if they had reconsidered.

If a participant did not attend follow-up clinic, the research nurses called the participant to re-organise their clinic appointment. If the participant was not contactable, their next of kin was contacted. Following this, a letter was sent to the participant's address. If the participant still did not attend clinic, the study team tried a number of methods to ensure the participant attended follow-up, including visiting the participant's home or place of work, using contacts in the community, visiting known places where the study participants socialised or went to church (Figure 8-2). If the participant was still not contactable or traceable following the above steps, they were considered lost to follow-up.

Figure 8-2. Nosipho, research nurse, on a field trip to find of one of the HOST 1 Study participants who had missed his follow-up appointment. He was homeless and his address was 'under the N1 motorway bridge, Woodstock, Cape Town'. The participant was located with his partner and subsequently came to all the rest of his clinic appointments until fracture union. Written consent was provided by the patient, spouse and research nurse to use these images.

8.4.4.4 Follow-up

The full visit assessment schedule for the study is provided in Table 8-2. Participants were followed up for 12 months from recruitment in total. X-rays were performed post-operatively to determine the fracture classification and at six weeks, three, six and nine months to assess union, and additionally out with this schedule if clinically indicated. Once union was confirmed by RUST score, no further x-rays were performed. All participants were seen in clinic at six months post-surgery. If a participant's fracture was confirmed to have united on RUST score at six months or sooner, they were followed up via telephone consultation at nine and 12 months. If they had not healed at six months, participants continued to have face-to-face follow-up in clinic at nine months. If they had healed, they were followed up at 12 months over the telephone. If they had still not healed at nine months and had a non-union

according to our outcome definitions, the participants were either offered surgery for revision of their non-union or, if there was still potential for bone healing without intervention in the view of the responsible orthopaedic consultant surgeon and myself, they were reviewed in clinic at 12 months with an x-ray. If at any time the responsible consultant surgeon felt that there was a need for further surgery to achieve union before nine months, surgery was offered, following a joint discussion with two consultant orthopaedic surgeons. (Figure 8-3)

Figure 8-3. Nosipho, research nurse, reviewing a participant in follow-up clinic and assessing their weight and height. Written consent was provided by the patient to use image.

Table 8-2. Full visit assessment schedule

Classification	Study measurement	Baseline	2 wks	6 wks	3 mths	6 mths	9 mths	12 mths
Predictor	HIV status/test	X						
Predictor	HIV investigations (CD4 count, viral load), if applicable	X				X		
Risk factor	ART status – if applicable	X			X	X	X	X
Risk factor	NSAID and steroid use	X	X	X	X	X	X	X
Risk factor	Blood tests (full blood count, renal function, albumin, Vitamin D)	X						
Risk factor	Socio-demographic characteristics & medical history	X						
Risk factor	Body Mass Index	X						
Risk factor	Bone mineral density assessment	X						
Intervention	Injury & treatment detail	X						
PROMS	Disability Rating Index	X			X	X	X	
2° outcome	Infection - ASEPSS score		X	X				
2° outcome	Infection – superficial surgical site infection		X	X				
2° outcome	Infection – deep surgical site infection		X	X	X			
2° outcome	Infection - late infection					X	X	X
Adverse events	Record complications/interventions		X	X	X	X	X	X
Concomitant treatment	Record rehabilitation		X	X	X	X	X	X

Main outcome	Radiographic union scale for the tibial fractures	X	X	X	X	X
2° outcome	Functional union		X	X	X	X
	Test performed once at baseline or within six weeks					
	Recorded once only, either at baseline or within three months					
	Not recorded if a participant was followed up over the phone					

Participants recruited and followed up at GSH had their BMD measured using a DEXA Heel Scanner, Calscan DXL (Figure 8-4). No Calscan DXL was available at TBH and although the scanner is portable, it did not leave GSH hospital for security reasons.

Figure 8-4. Participant undergoing measurement of their BMD with the Calscan DXL. Written consent was provided by the patient to use image.

Participants were reimbursed for their attendance at the follow-up clinic. This was in order for the participant to pay for their transport to clinic. The reason for this is due to the fact that the vast majority of participants enrolled were from low income households, lived far away from the hospitals and attending clinic commonly took the whole day, resulting in them missing a day's work. Furthermore, the research team's experience suggested that the likelihood of a participant attending clinic without this reimbursement was likely to be very low. At GSH and TSH the usual follow-up in trauma clinic is less than 40% following an orthopaedic operation (personal communication – Sithombo Maqungo and Nando Ferreira). Once the participant's fracture had healed there was no clinical reason for the participant to re-attend trauma clinic. This was within six weeks of injury for some participants. The

reimbursement was therefore used to allow participants to pay for their transport to clinic follow-up and compensate for any missed work to attend research follow-up. This is a method used by the majority of research teams undertaking clinical trials at the University of Cape Town or similar settings. It is also a method our research team has used successfully in the past. The amount and reimbursement schedule can be seen in Table 8-3.

Table 8-3. Participant reimbursement schedule.

Time point	Reimbursement amount – exchange rate 19.6.2019
Enrolment	150 Zar - £8.25
2 weeks	150 Zar - £8.25
6 weeks	150 Zar - £8.25
3 months	150 Zar - £8.25
6 months	200 Zar - £11
9 months – if required	200 Zar - £11
12 months – f required	200 Zar - £11

8.4.5 Study outcome

Primary study outcome:

- comparison of the cumulative incidence of delayed bone union between HIV-positive and -negative participants

Secondary study outcomes:

- comparison of the cumulative incidence of non-union between HIV-positive and -negative participants
- investigation of other risk factors associated with delayed bone union or non-union in HIV-positive and -negative adults, e.g. ART
- comparison of the cumulative incidence of superficial, deep and late wound infections between HIV-positive and -negative participants

8.4.5.1 Outcome definitions

The following are how the main outcomes of the study were defined.

Union:

• Radiological union on RUST score (score of three on at least three cortices in AP, lateral, medial or posterior cortex – a total of nine or more) within six months of surgery.(125), (126), (127), (128)

Delayed bone union:

• Impaired bone healing at six months on RUST score (RUST score < 9).(125), (126), (127), (128)

Non-union:

One or both of the following:

- Impaired bone healing at nine months on RUST score (RUST score < 9). (125), (126), (127), (128)
- Need for further surgery to achieve union (RUST<9) before nine months: decision made by two orthopaedic surgeons. Note that this definition was set at the beginning of the study and included in the study published protocol. However, no participant had confirmed non-union by this method. (125), (126), (127), (128)

Superficial wound infection

- Superficial surgical site infection (SSI)

The Center for Disease Control and Prevention definition of a 'superficial surgical site infection' is a wound infection involving the skin and subcutaneous tissue that occurs after 30 days of surgery (where day 1 = the procedure date).(402) (Appendix 13-4)

Deep wound infection

The Center for Disease Control and Prevention definition of a 'deep surgical site infection' is a wound infection involving the tissues deep to the skin that occurs within 30 days of injury (closed reduction of fracture) or 90 days (open reduction of fracture).(363) (Appendix 13-5)

Late implant infection

This describes any late wound breakdown (>30 days for closed reduction of fractures or >90 days for openly reduced fractures) or sinus formation, or unexplained late pain with associated radiological changes consistent with peri-implant infection.(388) This was determined on clinic follow-up for those still under review. Those participants who were under telephone review were called and assessed over the phone to report on their wound, using the described criteria. (Appendix 13-6)

Bone mineral density

- Normal – T score of <1 standard deviation (T score <−1) below the peak bone mass of a healthy young adult. European reference values were used since no comparison values for South Africa were available.
- Osteopaenia – T score of between 1 to 2.5 standard deviations (T score −1 to −2.5) below the peak bone mass of a healthy young adult.
- Osteoporosis – T score of >2.5 standard deviations (T score <−2.5) below the peak bone mass of a healthy young adult.

Injury Severity Score

- No polytrauma - Injury severity score of 15 or less on electronic Trauma Health Record
- Polytrauma - Injury severity score of 16 or more on electronic Trauma Health Record

Patient reported outcome measured (PROMS) Disability Rating Index (DRI) – Appendix 13-1

The DRI is a self-administered, 12-item Visual Analogue Scale questionnaire assessing the participants' own rating of their disability.(403) This measure was chosen as it addresses 'gross body movements' rather than specific joints or body segments. Therefore, it would facilitate the assessment of participants with different fractures and injuries of the lower limbs.

8.4.5.2 Determining the study outcome measures

Union

Bone healing was assessed using a validated X-ray scoring system – the radiological union scoring system for the tibia (RUST scoring system). (125), (126), (127), (128) Two independent reviewers (Maritz Laubscher and Pravesh Panchoo, both orthopaedic surgeons), blinded to HIV status, assessed radiological fracture union on radiographs at time points shown in Table 8-2. All patient identifiable information was removed from the x-rays prior to viewing them by the reviewers and no laboratory results were made available for them to assess HIV status. If there was a disagreement between the two reviewers regarding the outcome using the RUST score, a third reviewer (Professor Michael Held (MH), orthopaedic surgeon) independently reviewed the x-ray and assessed fracture union using the RUST score. Whichever RUST score outcome the third reviewer made (union or non-union), resulted in the final outcome decision for the participant.

The RUST score applies a score from 1-3 to the medial, lateral, anterior and posterior cortex of the bone (total 12). (123), (124) One indicates a fracture line with no bridging callus. Two indicates a fracture line with bridging callus and three suggests no fracture line plus bridging callus (see Chapter 3). If three cortices, out of four (medial, lateral, anterior and posterior), have a score of three (RUST = 9) then this fracture was classified as united.(125) If three out of four cortices did not have a score

of three (RUST ≤ 9) then this fracture was classified as delayed union (6 months) or a non-union (9 months) depending on the follow-up time point.

The zone of comminution was measured by a single orthopaedic surgeon (Maritz Laubscher) on the first post-operative x-ray using the graphics option of the computer x-ray software and a previously published technique.(404) The zone of comminution was measured from the most proximal part the fracture exited the bone, to the most distal point the fracture exited the bone. This method was used in a previous study undertaken in the same research department on similar study participant.(404) The reviewer was blinded to the HIV status of the patient. It is acknowledged that ideally, two reviewers who were blinded to the HIV status should have measured the fracture comminution and appropriate statistical analysis undertaken to determine inter-observer reliability should have been undertaken. However, due to time and resource restrictions this was not done. Therefore, due to potential inaccuracies and biases in the measurements of zone of comminution it was not included in the final analysis, but the results can be seen in Appendix 13-7.

Infection

When a participant was diagnosed with an SSI or DSI by the Principal investigator (Simon Graham), the participants had an assessment of their wounds in person by a single orthopaedic surgeon blinded to the HIV status (Martiz Laubscher – GSH, Nando Ferreira - TBH) and a photographs were taken.

Injury Severity Score

The injury severity score (ISS) was prospectively calculated by an electronic Trauma Health Record on admission hospital by the Trauma Care Team at both sites. (405) On the occasions that this was not completed prospectively on admission, one orthopaedic surgeon (Martiz Laubscher), who was blinded to the participants HIV status, completed the electronic Trauma Health Record for the participant retrospectively using the medical records and investigations from their admission.

Fracture classification

Fracture classification was determined by a single orthopaedic surgeon (Martiz Laubscher), blinded to HIV status, on post-operative x-ray. The AO classification system was used for the tibia and femur and the Winquist and Hansen classification system were used for the femur. Appendix 13-8, Appendix 13-9.

8.4.6 Completion of the study

The maximum length of time a participant was followed up for the study was 12 months. Participants who still needed follow-up assessments as part of the clinical management continued to be treated by the Trauma and Orthopaedic Surgery Unit, until their condition has stabilised to a point where no further medical intervention was required, and they were discharged.

8.4.7 Withdrawals

Participants who were recruited but declined to continue to take part in the study at any time were withdrawn without prejudice. A decision to decline consent or withdraw did not affect the standard of care the participant received and they continued standard hospital follow-up care.

Participants had two options for withdrawal:

1) Participants withdrew from completing any further questionnaires but allowed the study team to still view and retain, anonymously, any relevant hospital data that was recorded as part of normal standard of care, e.g. X-rays and further surgery information.

2) Participants withdrew wholly from the study, but data obtained up until the point

of withdrawal was included in the final analysis of the study, thereafter no further data was collected for that censored participant.

8.4.8 Adverse events

Serious adverse events (SAE) were defined as any serious untoward medical occurrence in a clinical study subject and which do not necessarily have a causal relationship with the treatment. These included any untoward and unexpected medical occurrence that: 'results in death', 'is life-threatening', 'requires hospitalisation or prolongation of existing in-patients' hospitalisation', 'results in persistent or significant disability or incapacity', 'is a congenital anomaly or birth defect' or 'any other important medical condition which, although not included in the above, may require medical or surgical intervention to prevent one of the outcomes listed'. These were recorded in on the electronic 'Redcap' database.

8.4.9 Sample size calculation

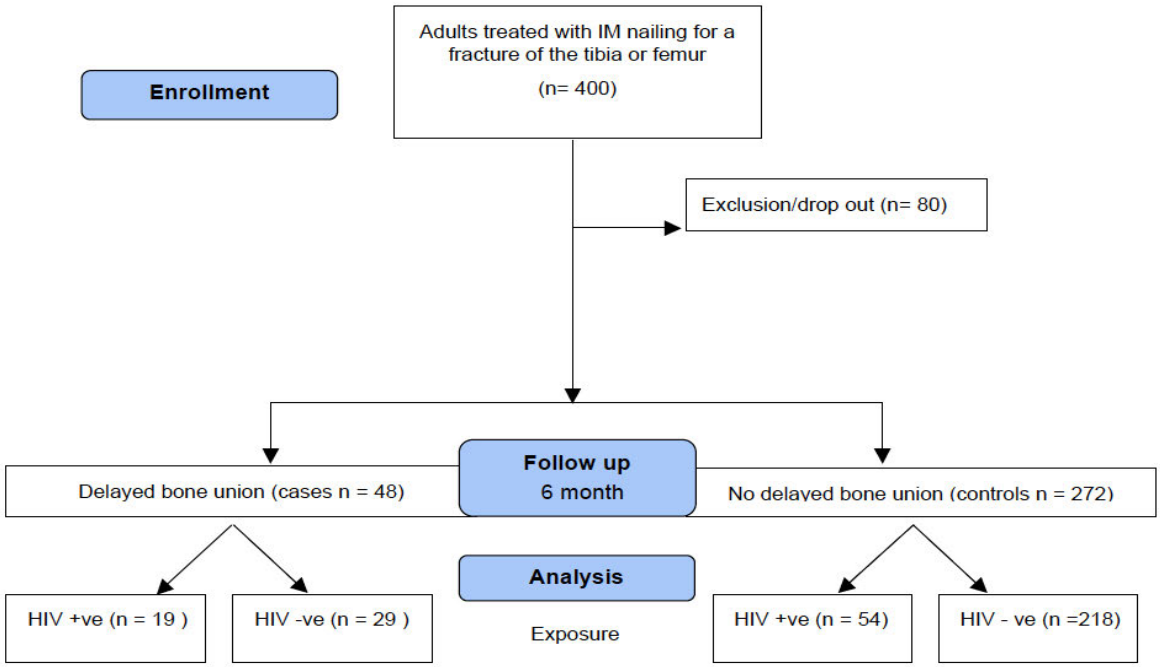
A previously established orthopaedic surgical register suggested that 400 participants were likely to undergo IM nailing of the tibia and femur at the two centres over the 14-month study period and would be eligible for inclusion: 80% (n=320) were assumed to be able to complete follow-up, giving an estimated overall loss to follow-up rate of 20%.

On the basis of previous research(114), (124), (126), (406), (407), it was estimated that 85% (n=272) of the 320 participants would have fracture union at six months (control), and 15% (n=48) would have delayed bone union (cases) (Figure 8-5).

Assuming that 20% of the controls would be HIV-positive (388), (368), the sample size would have 82.8% power to detect at least two-times difference in HIV prevalence in the cases compared to controls (from 20% to 40% at the 5% significance level).

For participants with more than one lower limb injury that met the inclusion criteria, each injury was included as an individual case and entered separately into the study. However, for the sample size calculation the participant was only counted as one participant, even if they had multiple IM nails. For example, if a participant had three lower limb IM nails, they were entered into the database as three different IM nails, with three different study numbers. However, they were classified as one individual participant when working towards the sample size calculation of 400 people.

Figure 8-5 Study sample size calculation



8.4.10 Statistical analysis

Distributions of baseline characteristics were summarised using means, medians, proportions and distributional measures (standard deviations and interquartile ranges) and tabulated and plotted and compared between exposure groups (HIV-positive vs. HIV-negative).

Analysis method and how the treatment effects were presented:

1. For the primary outcome (delayed union), a multivariable logistic regression model was constructed to estimate the odds ratio and 95% confidence interval for delayed union comparing between HIV-positive and HIV-negative participants and adjusting for important confounders identified *apriori* through construction of putative causal diagrams. A separate model was constructed for HIV-positive participants only to estimate the associations between HIV-associated predictors (e.g. CD4 cell count, viral load, ART use) on the outcome.

2. For the secondary outcome non-union, DSI similarly multivariable logistic regression model was constructed to estimate the odds ratio and 95% confidence interval for non-union comparing between HIV-positive and HIV-negative participants and adjusting for important confounders. Models were not constructed for secondary outcomes of SSI and late implant infection due to the low number of participants who experienced these outcomes.

When constructing the univariate and multivariable logistic regression models, confounding factors were identified from known factors that have been shown to clinically influence the outcome. For example, smoking is a well-established risk factor for delayed union and was therefore included in the delayed union multivariable logistic regression model. (163)

Confounders that had been identified to show little if any statistical significance in the study analysis or on testing the models with the parameter added, were not included in the modelling.

An example of how the models were constructed is summarised below. The models were initially built with established known confounding factors, plus HIV status. For delayed union the following confounding factors were added;

a) HIV Status
b) Gender
c) Age
d) Smoking
e) Open fracture
f) Fracture site
g) DSI

The models were then constructed, and addition factors were added to the models to assess if they made the model more accurate (e.g. post-operative haemoglobin). If this resulted in the fit of the model improving, as assessed by the likelihood ratio

test, then the factor was added to the model. If it did not, then it was excluded from the models.

Some of the enrolled participants had more than one tibia or femur fracture. Therefore, in the analysis, confidence intervals were adjusted for clustering.

Analysis of difference between continuous and categorical data not using logistic regression analysis, was assessed using t-test or chi-squared test respectively.

All statistical analysis was undertaken using 'R' statistical computing software.

8.4.11 Study timescale

The study schedule for the duration of recruitment and follow-up up was as follows:

- *September 2017 December 2018* – subject recruitment (baseline) took place at GSH and TBH (14 months)
- *October 2017- November 2019* – subject follow-up (up to 12 months) took place at GSH and TBH

8.5 Results: Descriptive analysis of HOST 1 study cohort

8.5.1.1 Recruitment

From September 2017 until December 2018, 638 participants underwent 683 IM nailings of the femur and tibia across the two study sites. All participants were screened and considered for inclusion in the study. 238 participants (241 IM nailings) did not meet the study inclusion criteria and were not enrolled (Figure 8-6). The predominant reason for participants not being enrolled was that the injuries were intertrochanteric femur fractures (88/241, 36.5%) and participant refusal to participate in the study (51/241, 21%). The full breakdown of the exclusion criteria and the number of participants excluded from enrolment can be found in Table 8-4.

Figure 8-6. Flow diagram of study population recruitment.

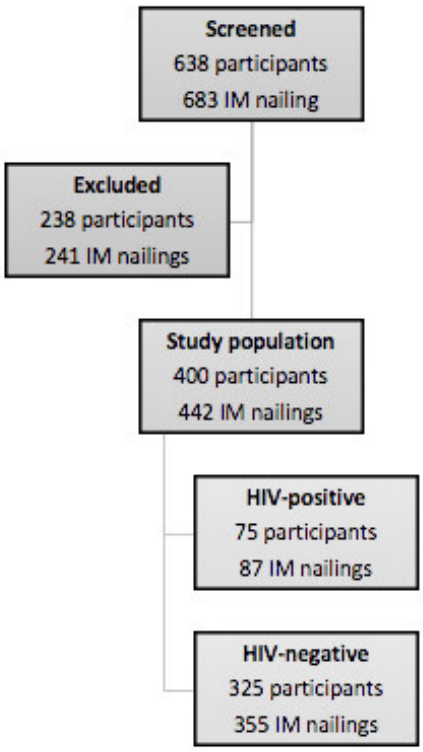


Table 8-4. Exclusion criteria and breakdown of the number of participants excluded from enrolment.

	GSH	TBH	Total
Participant <18 years	7	15	22
Participant refused to be enrolled	23	28	51
Head injury	5	20	25
Pathological fracture	1	1	2
Intertrochanteric femur fracture	86	2	88
Paraplegia	0	2	2
Dementia	2	3	5
Mental health disorder	0	10	10
Participant living out of area	4	7	11
Language barrier	5	4	9
Participant deemed too dangerous to enrol	0	4	4
Living on the streets and no contacts	0	8	8
Died prior to enrolment	0	3	3
Pre-surgical site infection	1	0	1
	134	107	241

8.5.1.2 Baseline characteristics

The main study cohort of 400 participants - who underwent 442 IM nailings - were enrolled in the study over a 14-month period from September 2017 until December 2018. Participants with 215 tibia and 227 femoral nails (right side: 242 and left side: 200) were enrolled. 361 participants had a single IM nail and 39 participants had more than one IM nail, all as part a single visit to the operating theatre on the same day (Table 8-5). The median age of the participants was 32.4 years (Interquartile range [IQR] 18-71 years) (Figure 8-7) and the majority of the participants were male, 313 (78.3%) vs female: 87 (21.7%).

Participants' median height was 1.70 meters (IQR: 1.37-1.88m) and median weight was 65 kilograms (IQR: 40-126 kg), giving a median body mass index (BMI) of 23.0 kg/m^2 (IQR:15.5 – 51.2) (Figure 8-8). As the age of the participant increased, the BMI increased. (Figure 8-9) Furthermore, men and smokers had a lower overall BMI (Figure 8-10, Figure 8-11).

There was a higher proportion of smokers compared to non-smokers – 225 (56.3%) vs 175 (43.3%). Almost all of the participants (98.22%) who smoked had been smoking for more than 12 months prior to enrolment.

The two study sites were tertiary referral trauma hospitals that receive referrals from surrounding district level hospitals. 24.5% (98/400) of the participants recruited were initially seen at a district level hospital before admission to GSH or TBH. All of the participants had their surgery at one of the two study sites. The median time taken to arrive at hospital following the injury was nine (4-24) hours.

The basic demographics and characteristics of the study participants are summarised in the table below (Table 8-5).

Table 8-5. Baseline characteristics of study population

Characteristic	Study cohort n=400	HIV-negative n=325	HIV-positive n=75	p value
Gender				
Male	313 (78.3)	262 (80.6)	51 (68)	0.030
Female	87 (21.7)	63 (19.7)	24 (32)	
Age (yrs: median, IQR)	32.36 (18-71)	31 (18-71)	35 (19-58)	0.080
BMI (kg/m^2: median, IQR)	23.02 (15.54–51.19)	22.9 (15.72-47.5)	23.31 (15.55-51.2)	0.720
Fracture site[a]				
Tibia	215 (48.6)	171 (48.2)	44 (50.6)	0.460
Femur	227 (51.4)	184 (51.8)	43 (49.4)	
Number of IM nails performed per participant				
1 nail	361 (90.3)	296 (91.0)	65 (86.7)	0.340
2 nails	37 (9.3)	28 (8.6)	9 (12.0)	
3 nails	1 (0.2)	1 (0.4)	0	
4 nails	1 (0.2)	0	1 (1.3)	
Drinks alcohol				
Yes	223 (55.8)	181 (55.7)	42 (56.0)	0.940
No	177 (44.3)	144 (44.3)	33 (44.0)	
Smoking				
Non-smoker	175 (43.8)	134 (41.2)	41 (54.6)	0.050
Smoker	225 (56.2)	191 (58.8)	34 (45.4)	
Transfer from district hospital				
Yes	98 (24.5)	74 (22.8)	24 (32.0)	0.100
No	302 (75.5)	251 (77.2)	51 (68.0)	

Time taken to arrive at treating hospital (Median:IQR, hours)	9 (4-24)	10 (4-24)	12 (6-18)	0.840
Patient reported outcome measure (PROMs)				
DRI pre-op (median: IQR)	0 (0-34.3)	0 (0-28)	0 (0-34.3)	0.090

BMI – body mass index

DRI- Disability rating index

CI – Confidence interval

IM- Intra medullary

IQR – Inter quartile range

a: n=442 / 355 / 87)

Figure 8-7. Age range of study participants

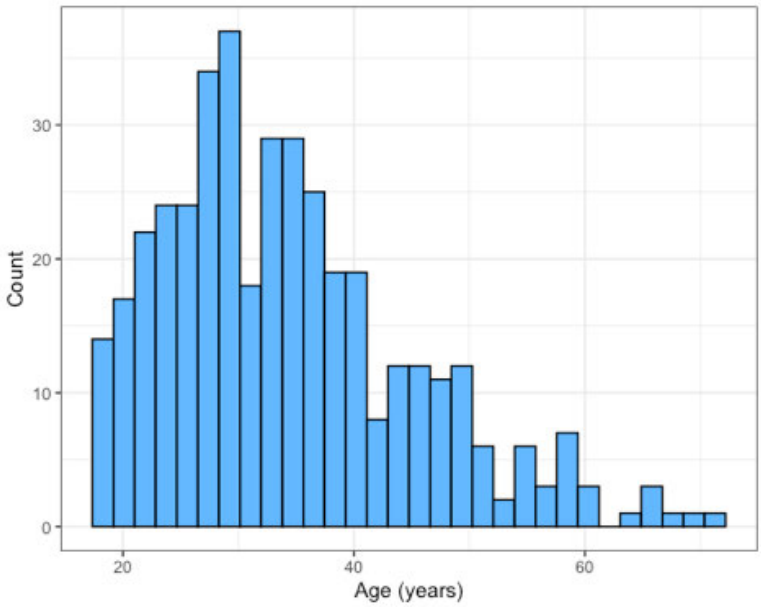


Figure 8-8. Body mass index range of the study participants

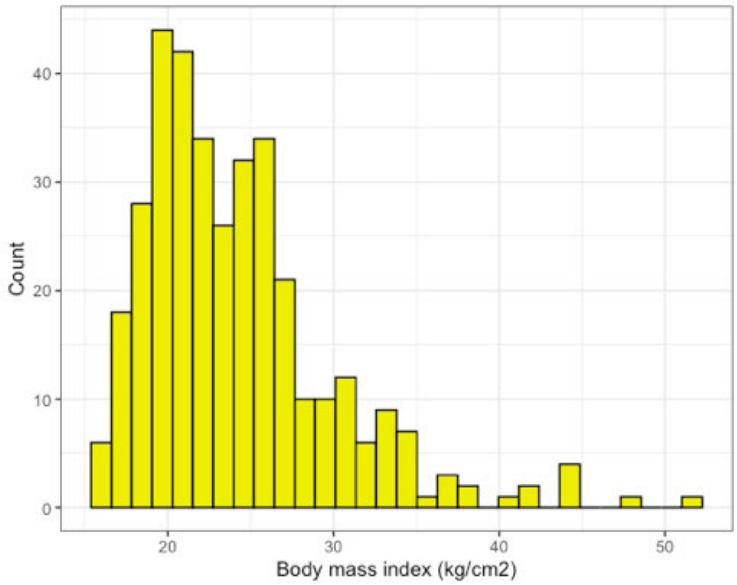


Figure 8-9. The relationship between age of participant and BMI

Line – represents linear regression model and 95% confidence intervals and same applies throughout thesis

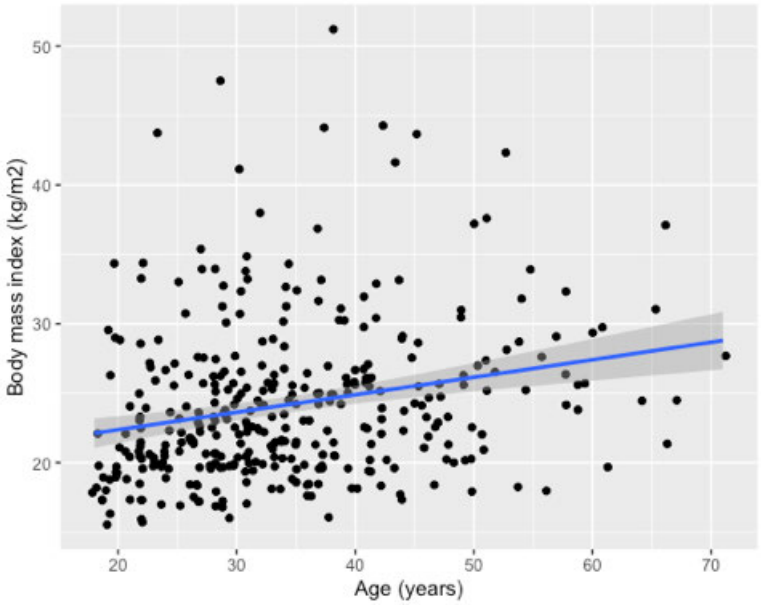


Figure 8-10. The relationship between sex and BMI of study participants

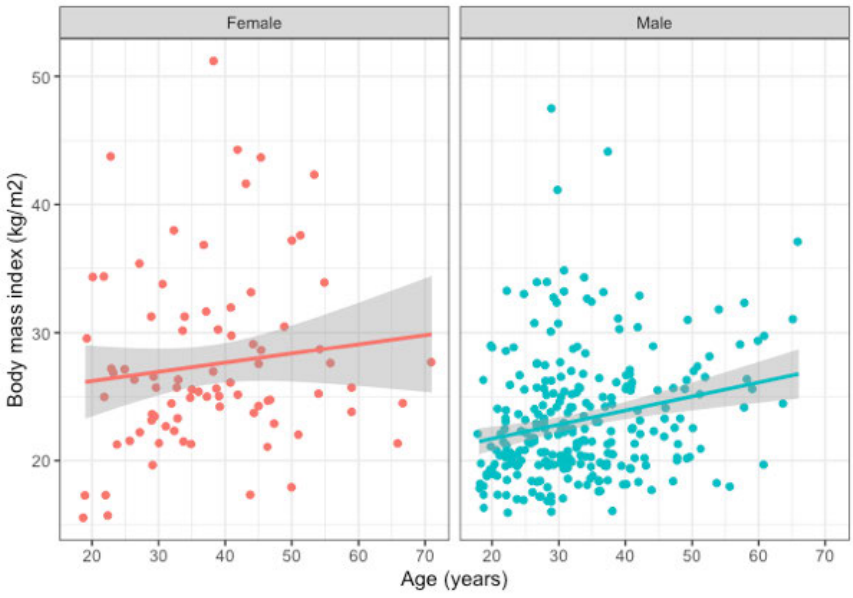
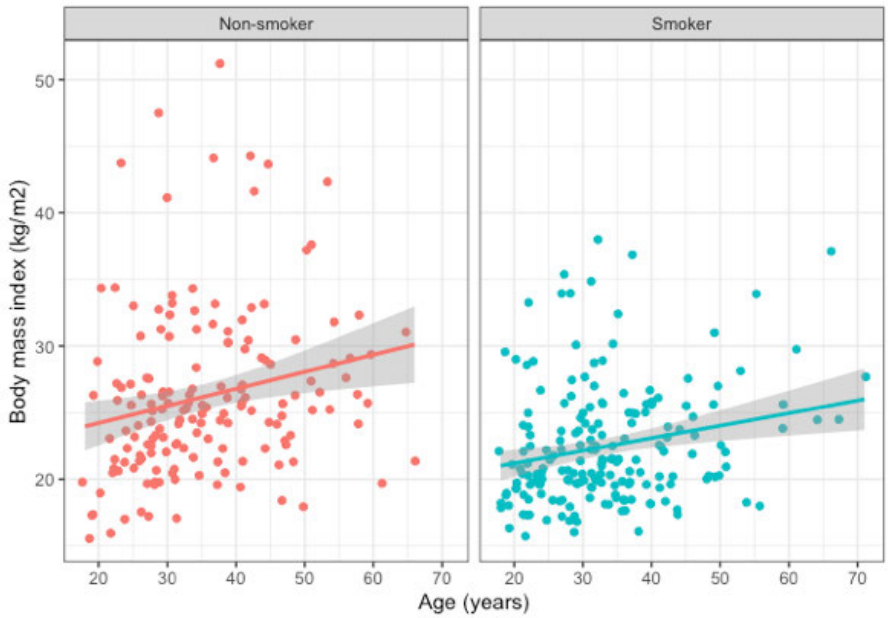


Figure 8-11. The relationship between smoking and BMI of study participants



8.5.1.3 HIV status

The prevalence of HIV in the study population was 18.8% (75/400 participants). The 75 HIV-positive participants underwent 87 IM nailings, giving an overall prevalence of HIV per IM nail of 19.7% (87/447) (Table 8-4). The HIV-positive participants were older than the HIV-negative participants –35 years (IQR 19-58) vs 31 years (IQR 18-71). (Figure 8-12) HIV-positive and negative participants had a similar BMI and HIV-positive males had a lower BMI than HIV-positive females (Figure 8-13). HIV prevalence was higher amongst smoker (HIV-positive 54% vs HIV-negative 41.2%) compared to non-smokers (p-value = 0.05). (Table 8-5).

Over 32% (24/75) of the HIV-positive participants were initially seen at a district level hospital prior to admission to one of the two study sites, compared to 22.8% (74/325) of HIV-negative participants, but this was not statistically significant (p-value = 0.100). However, this may suggest that a higher proportion of HIV-positive participants lived in areas away from the two tertiary referral hospitals. The district level hospitals commonly serve township areas in the Western Cape, with lower levels of social deprivation.

Figure 8-12. The median age of HIV-positive participants and HIV-negative at enrolment

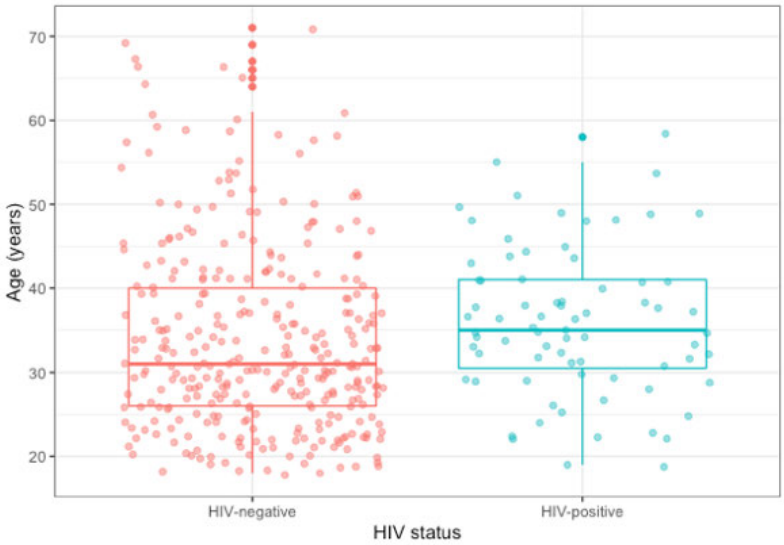
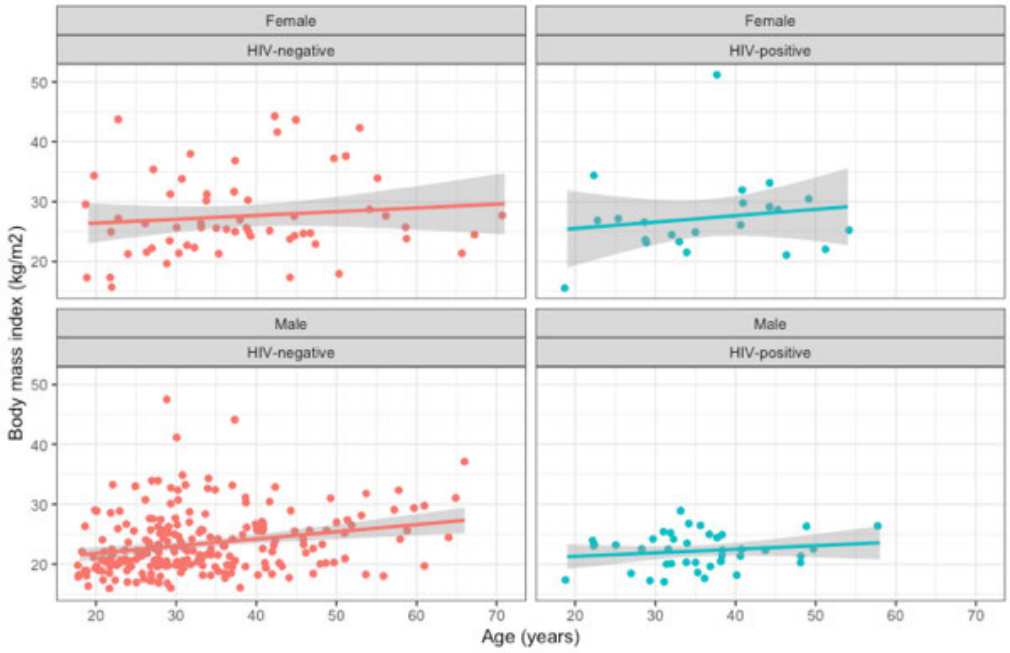


Figure 8-13. The relationship between BMI and age of participants at enrolment, stratified for sex and HIV status.



All 400 participants recruited had a HIV test. The majority of HIV-positive participants (42/75, 56%) knew their HIV diagnosis prior to enrolment in the study (Table 8-6). The rest of the participants had their diagnosis made during their admission (25/75, 33.3%) or within two weeks of their discharge (8/75, 10.6%). The median length of time a participant had a diagnosis of HIV was 1397(686-3565) days/3.8 (1.9-9.8) years.

42 out of 75 HIV-positive participants (56.0%) were taking ART on enrolment to the study and almost all of these participants were taking the same first line therapy - TDF, 3TC + ERV (38/42, 90.5%). As discussed in previous chapters, this is the current first line regiment for the treatment of HIV in the Western Cape of South Africa. Those participants who were on ART had been taking it for a median of 1732 (IQR 678-3568) days/4.7 (1.8-9.8) years.

The CD4 and viral load was measured for 65 out of the 75 HIV-positive participants within two weeks of surgery. The median CD4 count was 393 cell/mm^3 (IQR: 63-1145) and the median viral load was 2.13 log^{10} copies/ml (IQR: 1.3-4.62).

In the study population with HIV, older age correlated with lower viral loads and higher CD4 counts (Figure 8-14, Figure 8-16). This may reflect a compliance issue with the medication in the younger participants. This would suggest that compliance with medication is better in the older aged participants. This mirrors the literature, that has shown that higher proportions of older adults in sub-Saharan Africa are adherent to ART medication regimens compared with younger adults.(408)

The participants on ART on enrollment had a slightly lower baseline CD4 count (median CD4 344 cell/mm3 [IQR 264-566] vs median CD4 446 cell/mm3 [IQR 284-670] p-value=0.1) (Figure 8-15). However, those on ART had much lower viral load than ART naïve participants. (Figure 8-16).

The basic demographics and characteristics of the HIV study participants are summarised in the table below (Table 8-6).

Table 8-6. Baseline characteristic of HIV-positive participants

HIV parameter	n=75 (%)
HIV status[a]	
Positive	75 (18.8)
Negative	325 (81.2)
Diagnosis	
Before admission	42 (56.0)
On or during admission	25 (33.3)
After discharge	8 (10.6)
Number of IM nailing per participant	
1	65 (86.7)
2	9 (12.0)
3	0
4	1 (1.3)
Age at time of HIV diagnosis (median: IQR, years)	32.36 (17.46 – 48.23)
Length of time with HIV diagnosis (median: IQR, days)	1397 (686-3565)
Taking ART on admission	
Yes	42 (56.0)
No	33 (44.0)
Length of time taking ART therapy (median: IQR, days)	1732 (678-3568)
ART[b]	
TDF, 3TC + ERV	38 (90.5)
TDF, FTC + EFV	1 (2.4)
ZDV, 3TC + LPV/r	3 (7.1)
Age at the time of commencing ART[b] (median, IQR, years)	34.26 (17.46 – 48.23)

CD4+ count (cell/mm3) on admission (median, IQR)	393 (63-1145)
Viral load (cps/ml) on admission (Log^{10}, median, IQR)	2.13 (1.3-4.62)

ART – Anti-retroviral therapy

cps – copies

HIV – Human immunodeficiency virus

IQR – Inter quartile range

a: n=400

b: n=42

c: n=65

Figure 8-14. The relationship between CD4 count at baseline and age of HIV-positive participants.

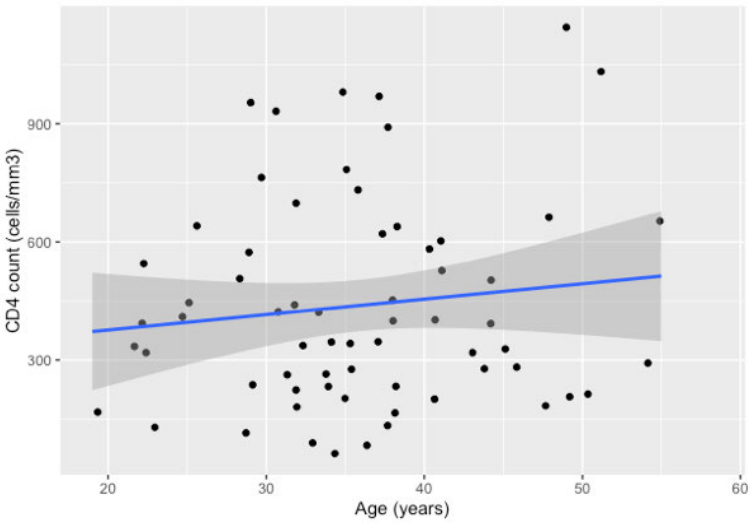


Figure 8-15. The relationship between CD4 count at baseline and age of HIV-positive participants: stratified by ART

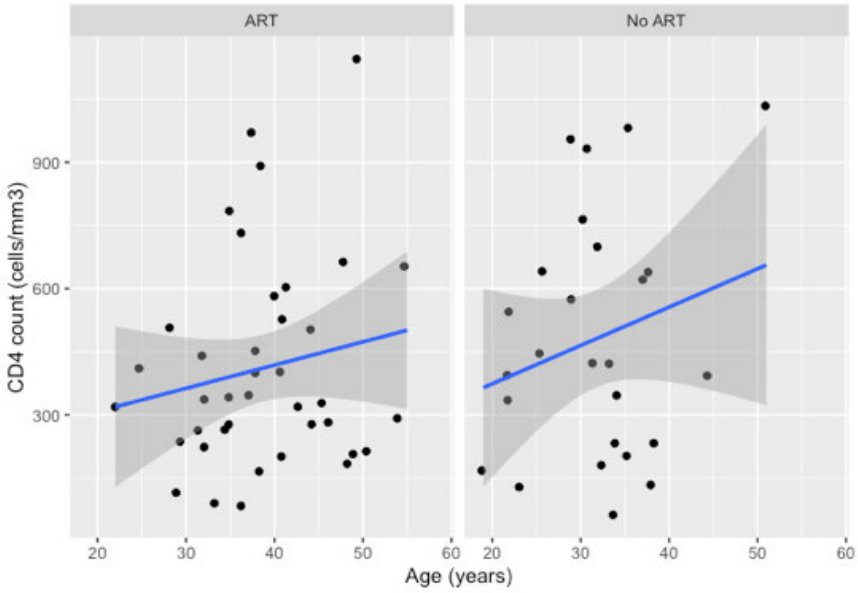


Figure 8-16. The relationship between viral load at baseline count and age of HIV-positive participants

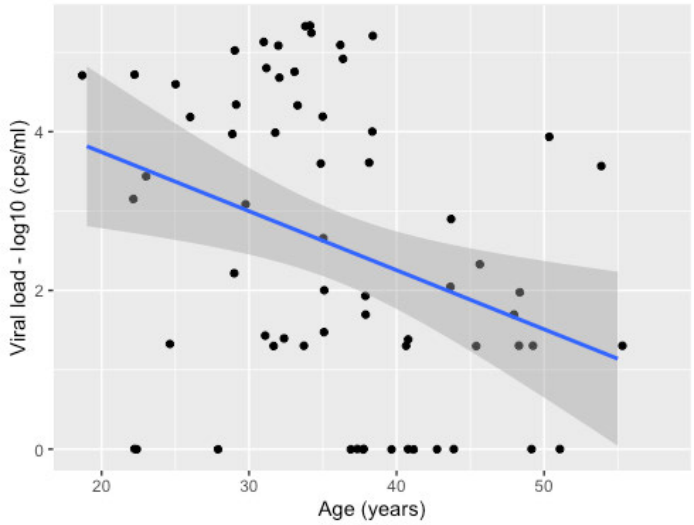
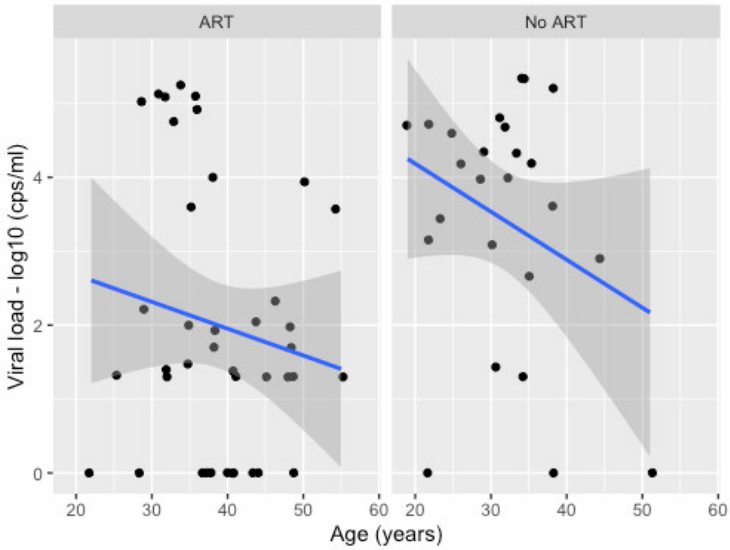


Figure 8-17. The relationship between viral load at baseline and age, stratified by ART



8.5.1.4 Place of residence

Out of the 400-participants enrolled in the HOST 1 Study, 350 participants provided a current fixed address in order to determine where the participants were living at the time of their injury. The majority of participants injured (342/350, 97%), lived in and around the Cape Town City Metropolitan Municipality (Figure 8-18). A further seven participants (Table 8-7) lived in the Western Cape and one participant lived in the Northern Cape but travelled back to Cape Town for their follow-up. A full breakdown of the location where participants lived, and maps of their Global Positioning System (GPS) home address can be seen in Figure 8-19, Figure 8-20, Figure 8-21.

Using GPS co-ordinates of participants addresses from enrolment and mapping out the approximate position of the Cape Flats, it was possible to determine that the highest density of the study population were living in the Cape Flats areas (250/342, 73.01% (Figure 8-22). The Cape Flats does not have an official designated border and the area is an approximate location of the boundaries.

Figure 8-18. City of Cape Town Metropolitan Municipality(409)

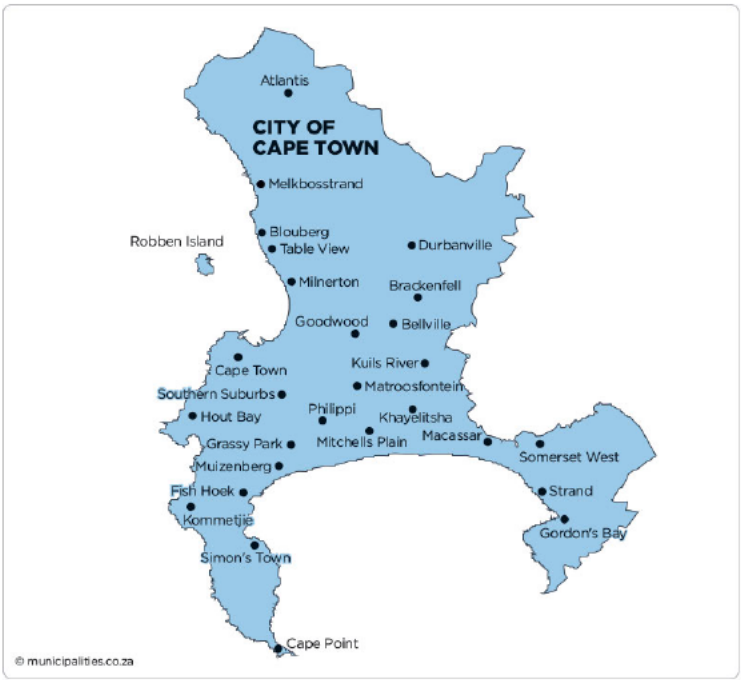


Table 8-7. Current home address at enrolment and during study follow-up of study population.

Living Address	**Participant (n=350)**
City of Cape Town - Western Cape	342
Klapmuts – Western Cape	1
St Helena Bay – Western Cape	1
Stellenbosch – Western Cape	2
Paarl – Western Cape	2
De Dooms – Western Cape	1
Kakamas – Northern Cape	1

Figure 8-19. Map of GPS co-ordinates of study populations throughout South Africa.

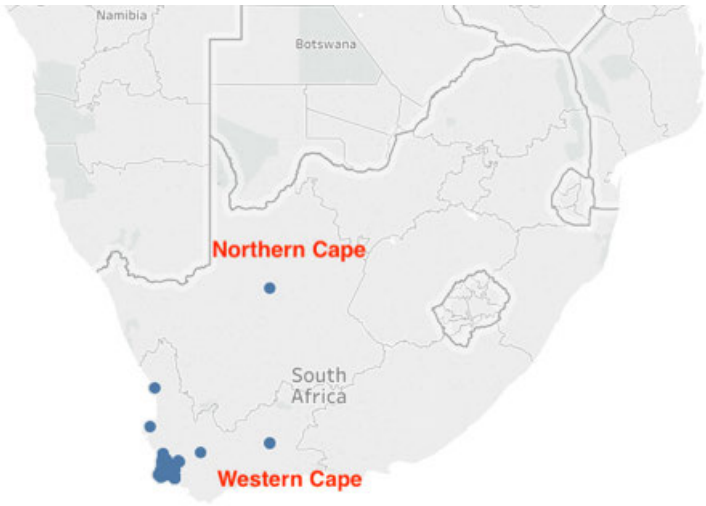


Figure 8-20 Map of GPS co-ordinates of study populations throughout the Western Cape.

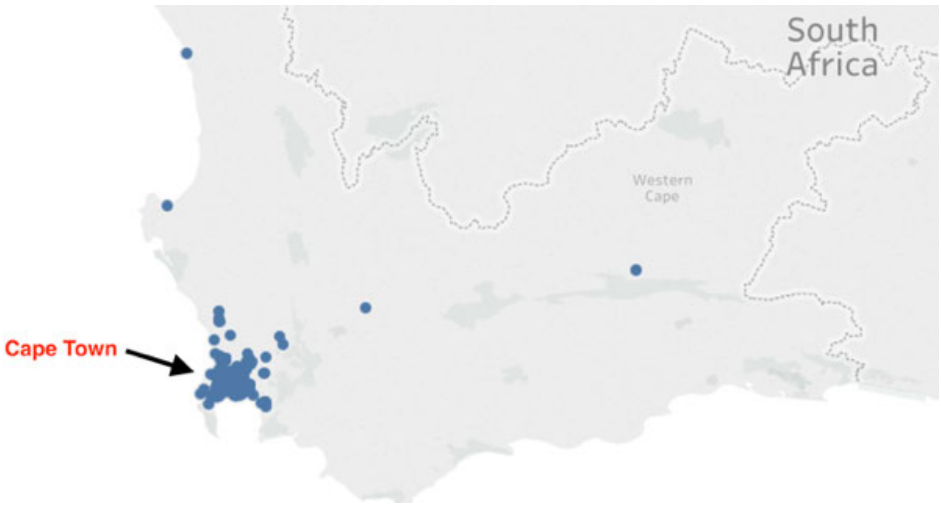


Figure 8-21. Map of GPS co-ordinates of study populations throughout the Cape Town and immediate surrounding area.

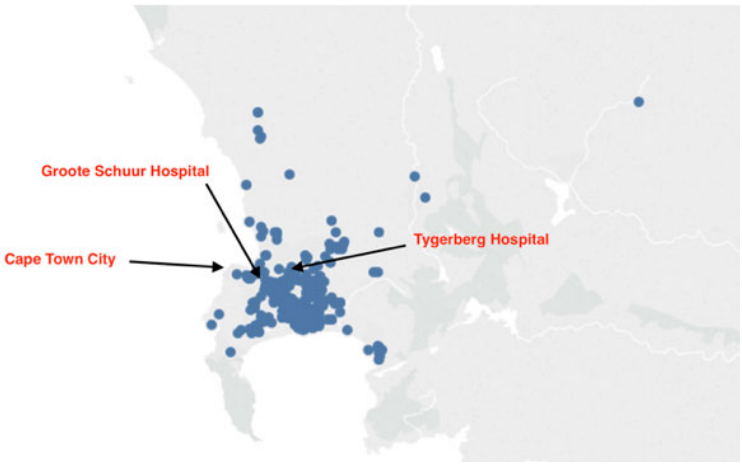
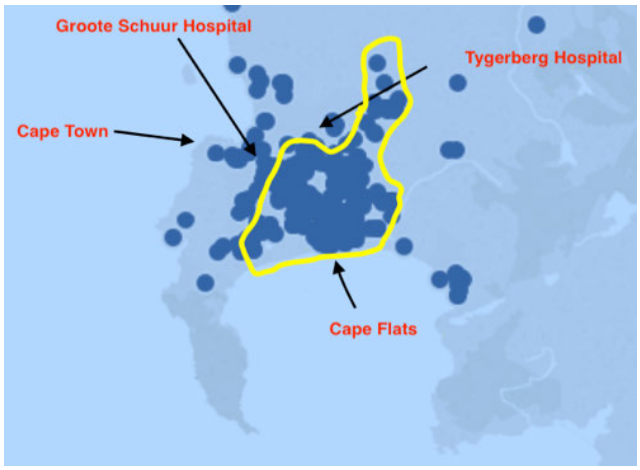


Figure 8-22. Map of the approximate outline of the Cape Flats area in relation to our study population.



8.5.1.5 Medical co-morbidities

The majority of the study population had no medical problems that increased their risk of developing non-union following a fracture. Five participants (1.25%) were diabetic. None of these participants were taking insulin, four were on oral tablets and one was diet controlled. A full summary of the medical risk factors for non-union can be found in Table 8-8.

16 patients (16 IM nails) had previously had a history of being treated for tuberculosis disease, with all reporting successful outcomes and were symptom free for at least six months before sustaining their injury. None of the patients were taking NSAIDs or steroids prior to their injury. A summary of the co-morbidities of the study population can be found in Table 8-8.

Table 8-8. Known medical co-morbidities of study population

Comorbidity	n=400 (%)
Peripheral vascular disease	
Yes	2 (0.5)
No	398 (99.5)
Rheumatoid arthritis	
Yes	2 (0.5)
No	398 (99.5)
Hypothyroid	
Yes	0
No	400 (100)
Renal impairment	
Yes	0
No	400 (100)
Diabetes	
Yes	5 (1.2)
No	395 (98.8) (4 tablets + 1 diet controlled)
Previous tuberculosis	
Yes	16 (4)
No	384 (96) (None active and all successfully treated)

A total of 181 participants (45.25%, n=400) had their BMD measured using a Calscan DXL Densitometer, all at GSH (Table 8-9). On measuring the 181 participants T-score, seven participants had a score that was diagnostic of osteoporosis (T-score < -2.5) (Figure 8-23, Figure 8-24).

Twenty seven out of 181 participants who underwent a measurement of their BMD were HIV-positive (15%)(Figure 8-23, Figure 8-24). There was a slightly higher

proportion of HIV-positive participants who were classified as osteoporotic (T score > 2.5) compared to HIV-negative participants (2/27 [7.4%] vs 5/154 [3.2%]), but no statistical significance was found between the groups (p-value=0.500). Although, it is noted that due to the low numbers, any valid conclusions are difficult draw from this. Overall, the BMD and T-scores were very similar in both the HIV-positive and negative participants (Table 8-9). This is surprising considering HIV and ART are known to be associated with osteoporosis.(282), (290)

As the age of the participants increased, the T-score declined, with males having higher T-scores compared to females (Figure 8-25, Figure 8-26). This was also true when stratifying T-score according to HIV status (Figure 8-27, Figure 8-28). Additionally, smokers had lower T-scores than none smokers (Figure 8-27), confirming already established knowledge about the increase risk of osteoporosis in smokers and females.(163), (410)

Table 8-9. Osteoporosis status of study population

Osteoporosis	**Study cohort n=181 (%)**	**HIV-negative n=154 (%)**	**HIV-positive n=27 (%)**	**P-value**
T-score osteoporosis classification (n=181)				
Normal > -1	105 (58.0)	89 (57.8)	16 (59.3)	0.500
Osteopenia -1 - -2.5	69 (38.1)	60 (39.0)	9 (33.3)	
Osteoporosis < -2.5	7 (3.9)	5 (3.2)	2 (7.4)	
BMD (g/cm^2: median, IQR)	0.49 (0.23-0.73)	0.49 (0.23-0.73)	0.49 (0.3-0.71)	0.700
T Score (median, IQR)	-0.8 (-3.8 - -2.4)	-0.8 (-3.8-2.4)	-0.8 (-3.2-2.1)	0.790
Z score (median, IQR)	-0.6 (-3.2 - -2.4)	-0.55 (-2.8-2.4)	-0.8 (-3.2-2.4)	0.540

BMD – bone mineral density

T score – compares BMD to that of a 30-year-old adult

Z score – compares BMD to that of an adult and sex matched individual

Figure 8-23. Bone mineral density of study population

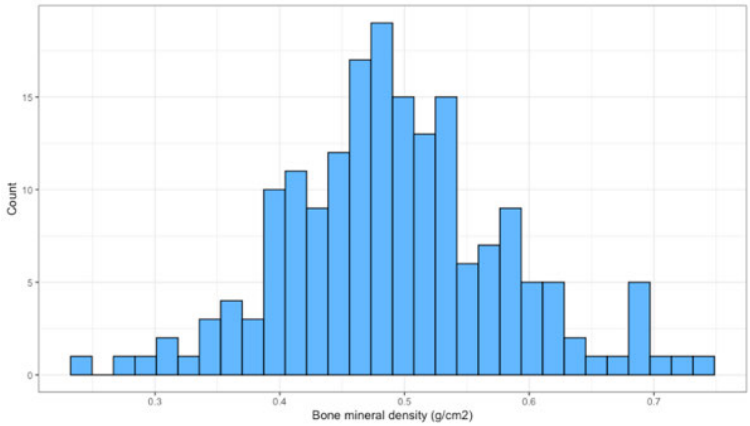


Figure 8-24. T-score of study population

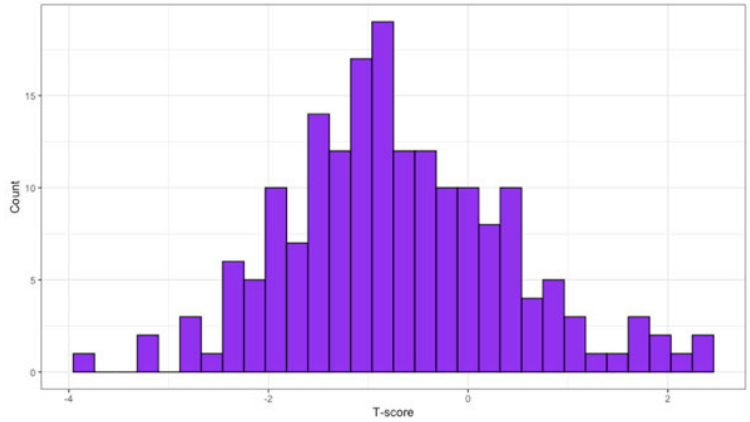


Figure 8-25. Relationship between T-score and age

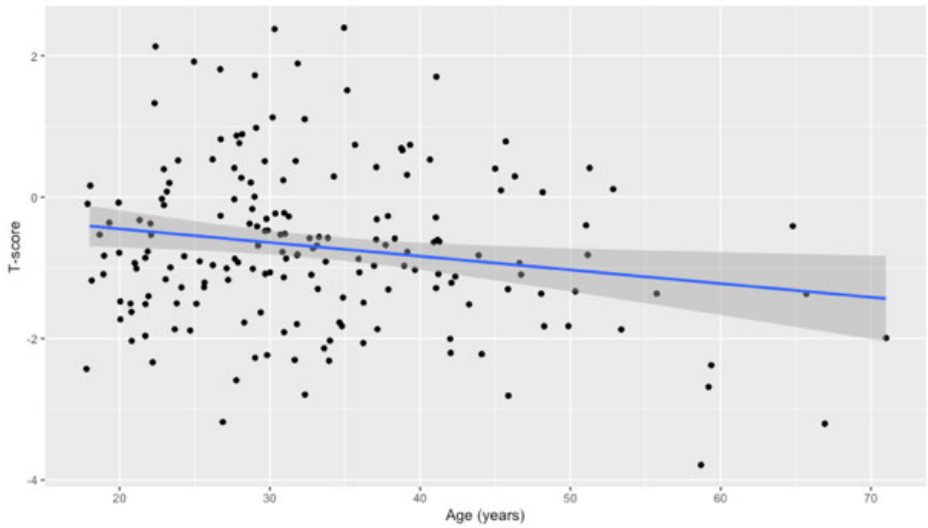


Figure 8-26. The relationship between T score and age, stratified by sex

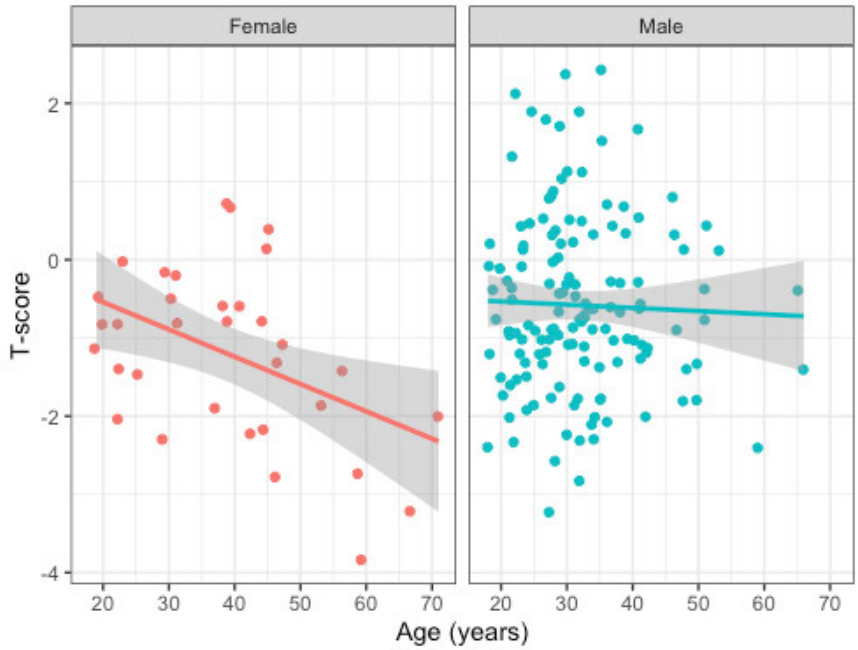


Figure 8-27. The relationship between T score and age, stratified by smoking status

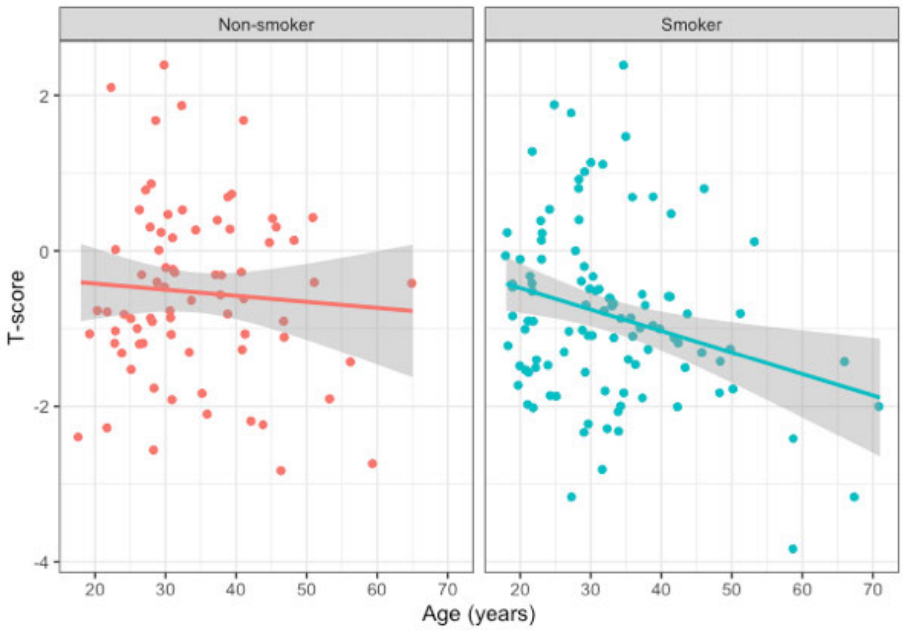


Figure 8-28. The relationship between T-score and age, stratified by HIV status

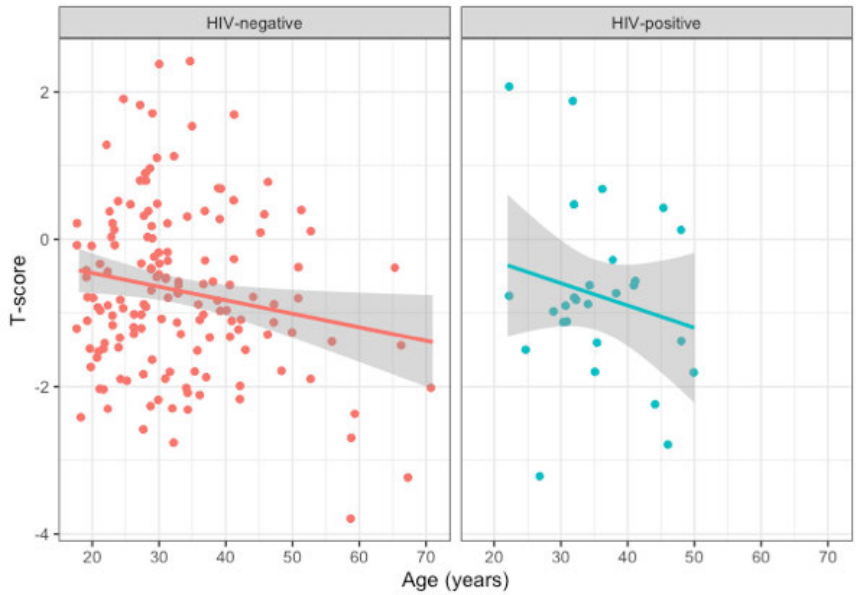
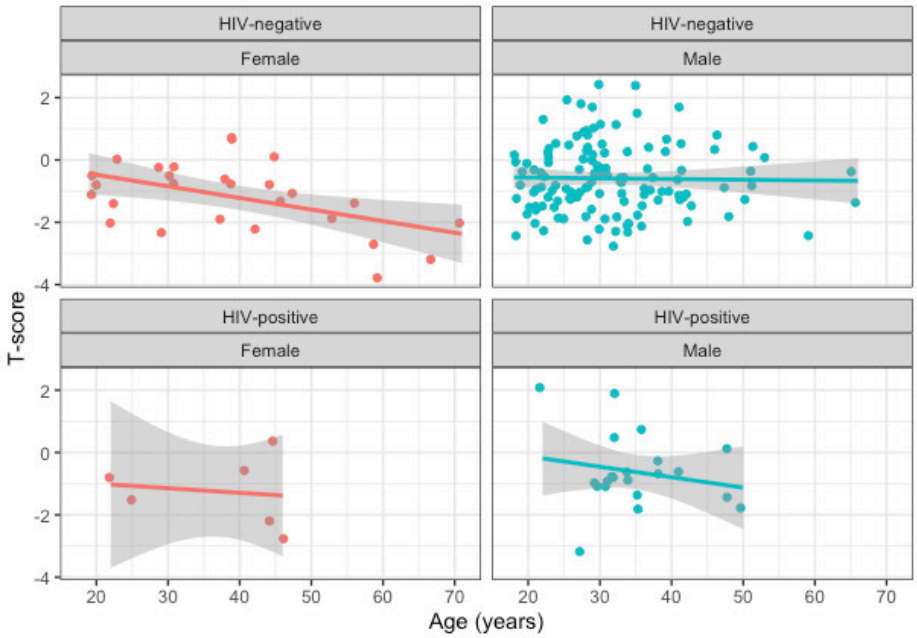


Figure 8-29. The relationship between T-score and age, stratified by HIV status and sex



8.5.1.6 Household and socioeconomic characteristics

The level of completion of secondary school education in the Western Cape is 65.4% (411), compared to 71.5% (286/400) in the study population (Table 8-4). The unemployment rate in the Western Cape is reported to be 29.1%. (412) However, between the age of 15-24 years it is 55.2% and decreasing to 32.4% in ages 25-34 years.(412) In the study population the unemployment rate was much higher than the regional average, at 52.5 % (210/400) with a median age of 32.36 years (18-71 years).

The percentage of households connected to an electricity supply from the mains in the Western Cape has increased from 76.7% in 2002 to 84.4% in 2017.(413) The percentage of households that used electricity for cooking increased from 57.5% in 2002 to 75.9% in 2017.(413) Whereas, over 97.65% (391/400) of participants had access to mains electricity and 94.8% (379/400) used electricity as the primary method of cooking in the study population. Only 74% (296/400) of the study population had access to piped water to their home, compared to 88.6% of South Africans.(414)

Currently, just over four-fifths (80.1%) of South African households lived in formal dwellings, followed by 13.6% in informal dwellings, and 5.5% in traditional dwellings.(413) The majority of study participants lived in structured formal housing (373/400, 93.3%), as measured by the material made up of the floor to their homes. Although higher levels of formal housing and access to electricity are higher than the regional average. The fact access to piped water are lower and the level of education and employment is much lower, the socioeconomic level of the study cohort as a whole appears lower than the regional average.

The HIV-positive cohort had a higher crowding index (1.5 [IQR: 0.25-4] vs 1.33 [IQR:0.25-7]), derived from the total household size divided by the number of living

rooms, compared to the HIV-negative participants, although not statistically significant (p-value= 0.72). Higher crowding index has been shown to correlate with socioeconomic status.(415) The proportion of parameters, including presence of a flushing toilet in the home (63/75, 84% vs 308/32, 94.8%), piped water to own home (40/75, 53.3 vs 256/325, 78.7%) and access to mains electricity (69/75, 92 % vs 322/325, 99.1%) were all lower in the HIV-positive compared to the HIV-negative cohort. Regarding access to a flushing toilet and piped water to the participants homes, this was shown to be statistically significant between the two groups (p-value=0.009, p-value=0.001). Furthermore, the level of education was lower in HIV participants and the level of unemployment was also higher (46/75, 61.3% vs 164/325, 50.5%) (Figure 8-30, Figure 8-31). This is coupled with earlier findings that a higher proportion of the HIV-positive participants first sought medical treatment at a district level hospital (38/75, 50.7% vs 60/325, 18.5%), suggesting that the socioeconomic status of the HIV-positive cohort is lower than the HIV-negative group. Although, it is noted the only parameters that resulted in a significant difference between HIV-positive and negative participants was the access to piped water and a flushing toilet and there was no formal poverty score undertaken.

A summary of the socioeconomic characteristics of the study population can be seen in Table 8-10.

Table 8-10. Socioeconomics of study population

	Study cohort n = 400, %	HIV-negative n = 325, %	HIV-positive n = 75, %	P value
Number of people living in household (median, IQR)	4 (1-18)	4 (1-11)	5(1-18)	0.069
Number of living/dwelling rooms in household[a] – kitchen and bathrooms not included (median, IQR)	3 (0-11)	2 (0-11)	2(0-6)	1.0
Crowding index[i] (median, IQR)	1.33 (0.25-7)	1.33 (0.25-7)	1.5 (0.25-4)	0.720
Toilet				
Flush	371 (92.8)	308 (94.8)	63 (84.0)	0.009
Chemical	9 (2.3)	5 (1.5)	4 (5.3)	
Pit/latrine	7 (1.7)	5 (1.5)	2 (2.7)	
No sanitation facilities	13 (3.2)	7 (2.2)	6 (8.0)	
Water supply				
Piped to dwelling	296 (74.0)	256 (78.8)	40 (53.3)	0.001
Piped to yard	82 (20.5)	56 (17.2)	26 (34.7)	
Public tap/standpipe	22 (5.5)	13 (4.0)	9 (12.0)	
Borehole	0	0	0	
Stream	0	0	0	
Principal cooking fuel				
Electricity	379 (94.8)	310 (95.4)	69 (92.0)	0.500
Gas	15 (3.7)	11 (3.4)	4 (5.3)	
Wood	0	0	0	
Coal	0	0	0	
Other	6 (1.5)	4 (1.2)	2 (2.7)	
Access to mains electricity to home				
Yes	391 (97.65)	322 (99.1)	69 (92)	0.500
No	9 (2.25)	3 (0.9)	6 (8)	

Material making up floor of household				
Natural/sand/dirt	11 (2.8)	9 (2.7)	2 (2.7)	1.000
Rudimentary /wood/planks	15 (3.8)	12 (3.8)	3 (4)	
Finished/carpet/tiles	373 (93.3)	303 (93.2)	70 (93.3)	
Homeless	1 (0.3)	1 (0.3)	0	
Level of education				
Never attended	0	0	0	0.200
Primary	49 (12.3)	35 (10.8)	14 (18.7)	
Secondary	286 (71.5)	234 (72.0)	52 (69.3)	
Higher	62 (15.5)	53 (16.3)	9 (12.0)	
Postgraduate	3 (0.8)	3 (0.9)	0	
Employment				
No formal employment (incl.	210 (52.5)	164 (50.5)	46 (61.3)	0.300
student)	96 (24.0)	80 (24.6)	16 (21.3)	
Unskilled	77 (19.3)	64 (19.7)	12 (16.0)	
Skilled	17 (4.3)	16 (4.9)	1 (1.3)	
Professional				

i Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included)

IQR – Inter quartile range

Figure 8-30. Participants' occupation according to HIV status

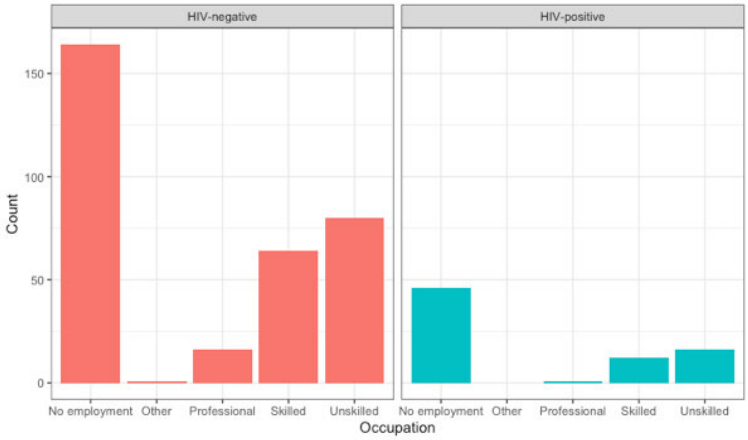
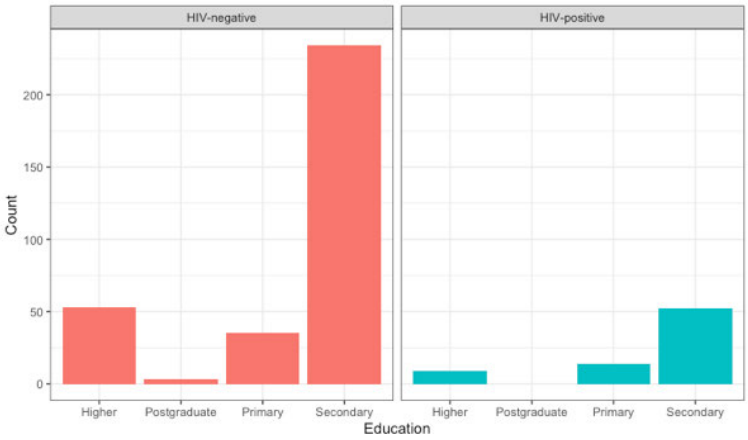


Figure 8-31. Participants' level of education according to HIV status



8.5.1.7 Blood parameters

The majority of the participants enrolled had pre-operative (310/400, 77.5%) and post-operative haemoglobin (387/400, 96.8%) measured. The HIV-positive participants had a lower median starting pre-operative haemoglobin (11.5 [6-14.9] vs 12.5 [5.4-17.8] g/dL) (Figure 8-32), but a lower post-operative haemoglobin drop (1.35 vs 2 g/dL)(Figure 8-33, Figure 8-34, Figure 8-35). There was a statistically significant difference in the pre and post-operative haemoglobin in the HIV-positive, compared to the HIV-negative participants (p vale = 0.001 and 0.005). As the age of the HIV-positive participants increased, the lower the level of post-operative haemoglobin. This is in contrast to the increase in haemoglobin in the HIV-negative participants (Figure 8-36).

HIV-positive participants had a significant difference in albumin compared to HIV-negative individuals (36 vs 44 g/L, p-value = 0.02). Albumin has been used as an indicator of nutritional status. However, it has been shown to poorly correlate with nutritional parameters and cannot be used independently to suggest any difference in the nutritional status between the HIV-negative and positive participants .(416)

As with post-operative haemoglobin, the older the HIV-positive participant, the lower the vitamin D and albumin measurement, whereas in the HIV-negative participants these levels remained relatively static throughout all age groups (Figure 8-41, Figure 8-42).

Regarding the duration of surgery, the post—operative haemoglobin was similar in participants whose procedure lasted less than one hour (10.12 g/dL) or took between two to four hours (10.18 g/dL)(Figure 8-43). However, once the procedure took longer than four hours, there was an unusually a higher post-operative haemoglobin in these individuals. These results are likely to be skewed by the relatively low number of patients whose procedure lasted over four hours (six nailing's).

Table 8-11. Blood results of study population

Blood parameter	Study cohort	HIV-negative	HIV-positive	P - value
Pre-operative				
Haemoglobin[a] (g/dL) (median/IQR)	12.3 (5.4-17.8)	12.5 (5.4-17.8)	11.5 (6-14.9)	0.001
Post-operative				
Haemoglobin[b] (g/dL) (median/IQR)	10.4 (5.5-18.5)	10.5 (5.5-18.5)	10.15 (5.9-14.9)	0.005
White blood cells[c] (x 10^9 / L) (median/IQR)	8.49 (2.81-46)	8.59 (3.2-44.19)	8.15 (2.81-25.3)	0.150
Creatine[d] (umol/L) (median/IQR)	61 (30-137)	72 (31-137)	58 (30-101)	0.010
Urea[e] (mmol/L) (median/IQR)	3.9 (1.2-46)	5.1 (1.6-46)	3.5 (1.2-7.4)	0.010
Vitamin D[f] (nmol/L) (median/IQR)	45 (7.5-136)	45.1 (7.5-136)	41.25 (11.6-123.8)	0.850
Albumin[g] (g/L) (median/IQR)	37 (20- 54)	44 (20-54)	36 (22-48)	0.020

IQR – Inter quartile range

a: n=310, n -ve = 249, n +ve = 61

b: n=387, n -ve= 315, n +ve = 72

c: n=390, n -ve= 318, n +ve=72

d: n=392, n - ve=319, n +ve=73

e: n=377, n -ve=309, n +ve = 68

f: n=379, n -ve=310, n +ve= 69

Figure 8-32. Pre-operative haemoglobin of study population

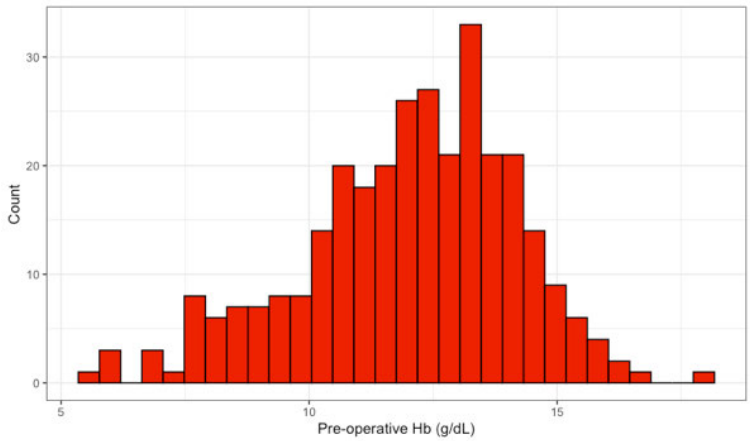


Figure 8-33. Post-operative haemoglobin of study population

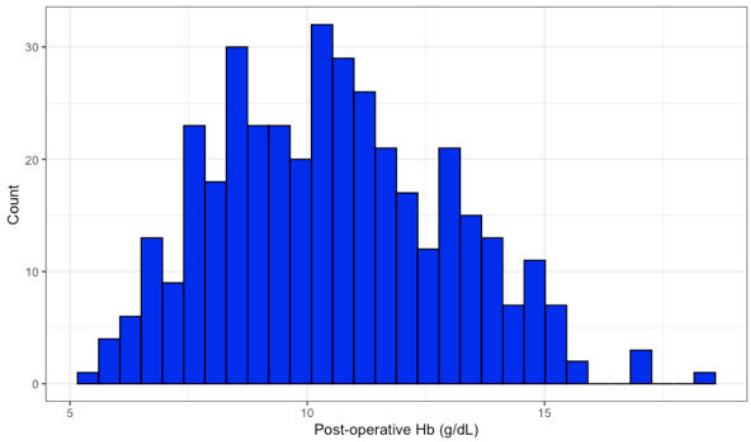


Figure 8-34. Relationship between pre-operative and post-operative haemoglobin accoridng to HIV status

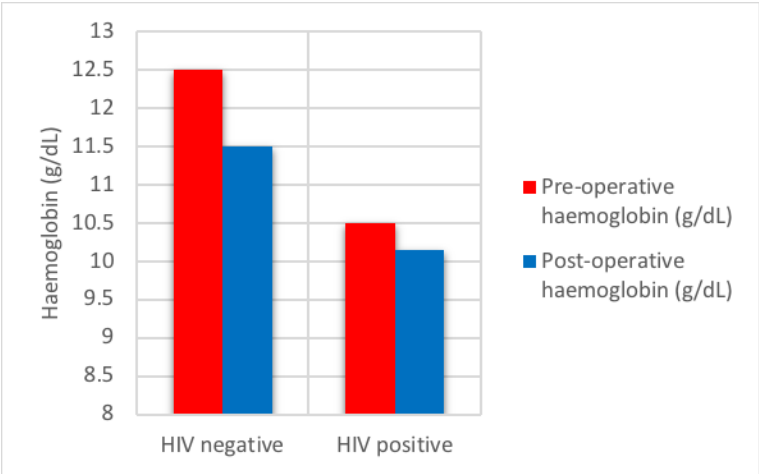


Figure 8-35. The relationship between post-operative haemoglobin stratified according to HIV status

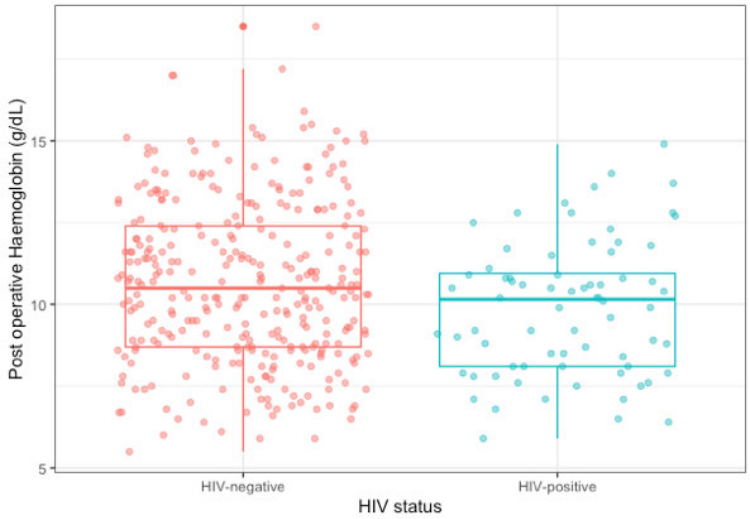


Figure 8-36. The relationship between post-operative haemoglobin and age, stratified according to HIV status

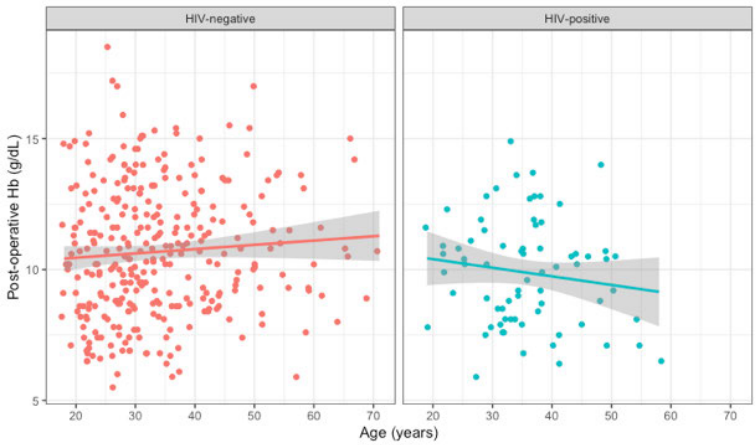


Figure 8-37. Post-operative vitamin D of study population

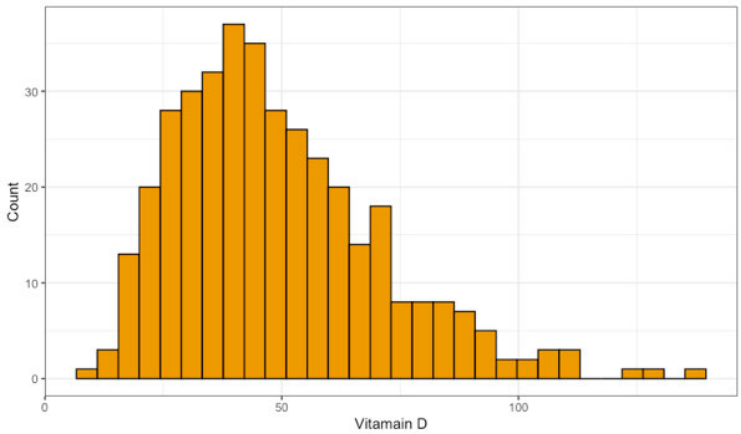


Figure 8-38. Relationship between pre-operative and post-operative vitamin D level according to HIV status

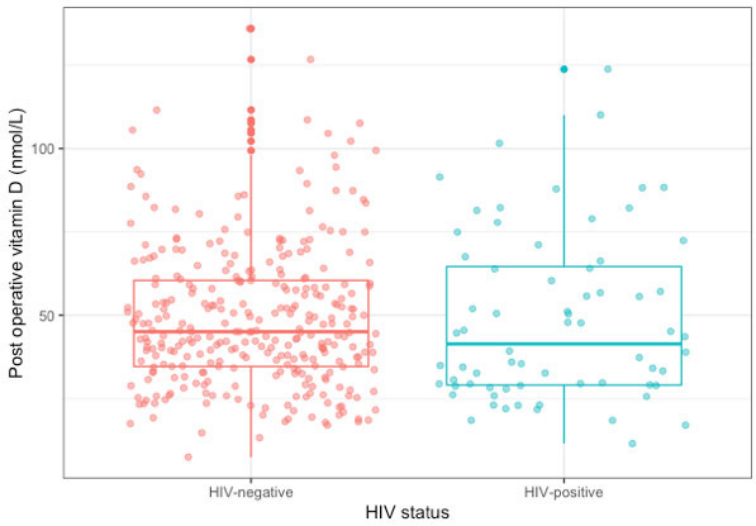


Figure 8-39. Post-operative albumin of study population

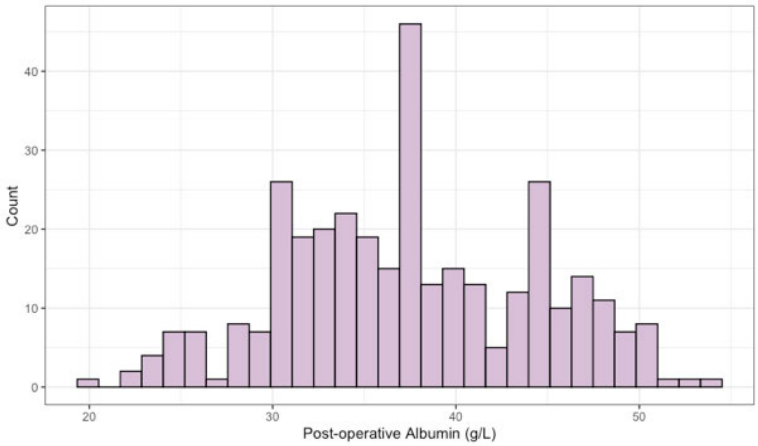


Figure 8-40. The relationship between post-operative albumin level according to HIV status

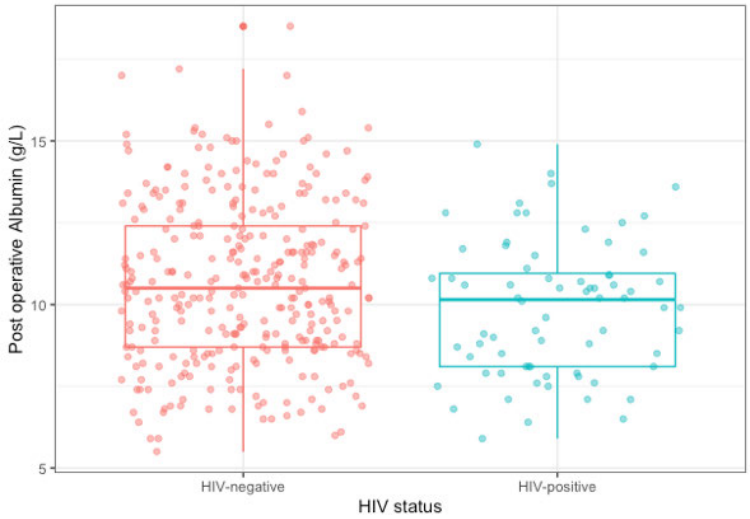


Figure 8-41. The relationship between vitamin D and age, stratified according to HIV status

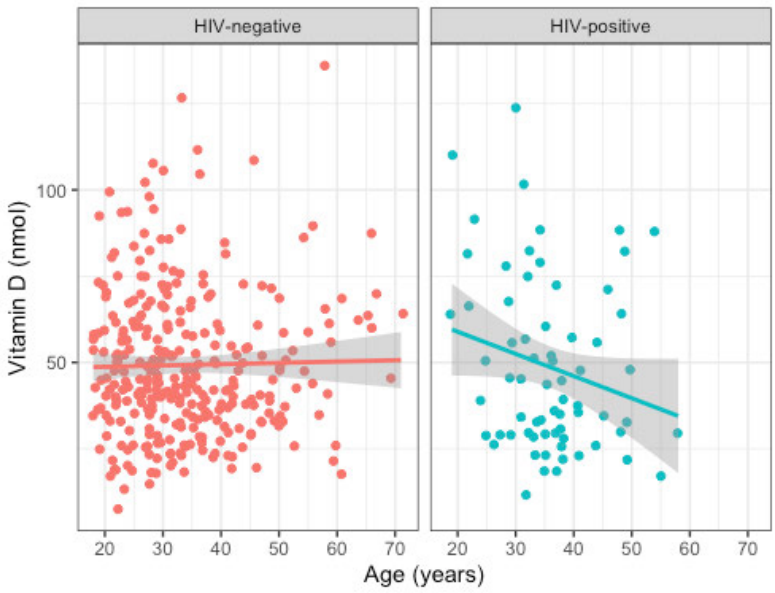


Figure 8-42. The relationship between albumin and age, stratified according to HIV status

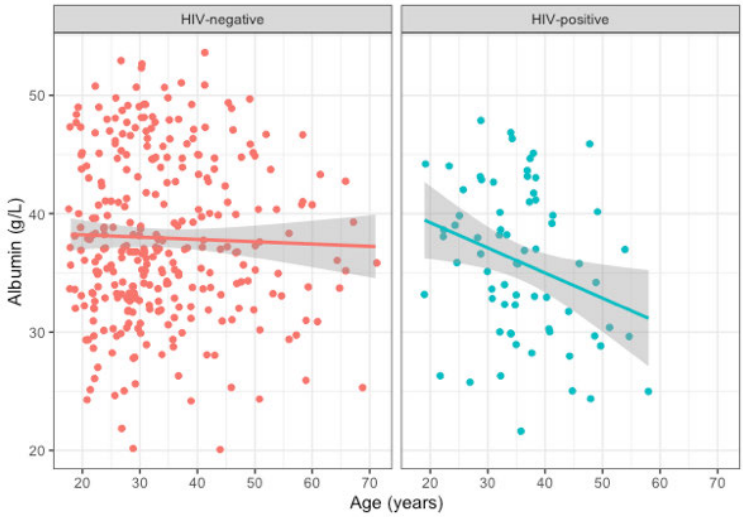
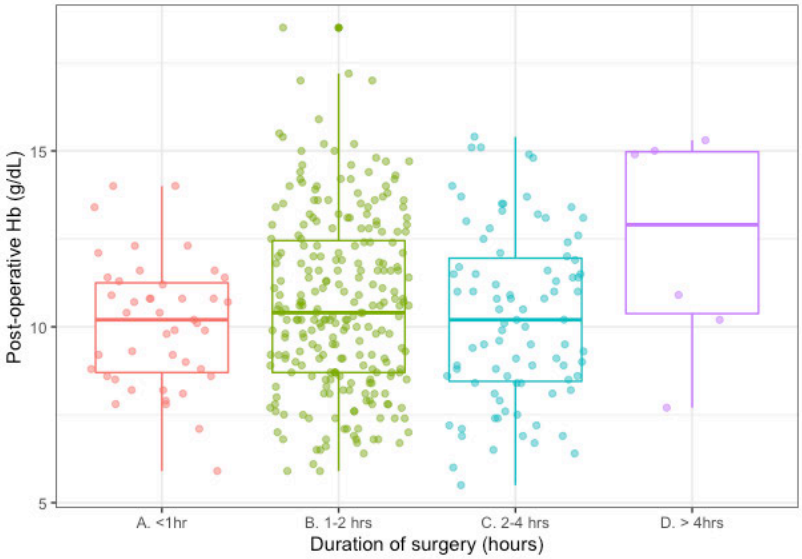


Figure 8-43 The relationship between post-operative haemoglobin and duration of surgery.



8.5.1.8 Mechanism of injury

A full breakdown of how the mechanism of injury was defined can be found in Appendix 13-10. The main mechanism of injury in the whole study population was due to road-related injuries.(Table 8-12) 62.5 % (276/442 IM nailings) of injuries were due to participants being hit by a car whilst a pedestrian or being in a road traffic collision in a vehicle. 21.5% (95/442 IM nailings) of injuries were due to GSWs, with the majority GSW being low velocity injuries (92/442 IM nailings).

There was a higher proportion of GSWs in the HIV-negative participants (23.9% [85/355] vs 11.15 % [10/87]). However, there was a much higher proportion (49.4% (43/442) vs 32.7% (116/442)) of participants injured after being hit by a vehicle as a pedestrian, compared to HIV-negative participants. Additionally, more of the injuries in the HIV participants were due to road-related injuries (70.1% (61/442) vs 60.6% (215/442)).

Table 8-12. Mechanism of injuries of study population

Mechanism of injury	Study cohort n = 400 (%)	HIV-negative n = 355 (%)	HIV-positive n = 87(%)	p-value
Low energy	28 (6.3)	21 (5.9)	7 (8.1)	0.002
High energy	20 (4.5)	17 (4.9)	3 (3.5)	
MVA: Car/motorbike/truck	117 (26.5)	99 (27.9)	18 (20.7)	
MVA – pedestrian	159 (36.0)	116 (32.7)	43 (49.4)	
Gunshot wound	95 (21.5)	85 (23.9)	10 (11.5)	
Low energy	92 (96.8)	82 (96.5)	10 (1.00)	
Medium energy	3 (3.2)	3 (3.5)	0	
High energy	0	0	0	
Sharp	2 (0.5)	0	2 (2.3)	
Blunt	16 (3.6)	13 (3.7)	3 (3.5)	
Crush	5 (1.1)	4 (1.1)	1 (1.2)	

MVA: Motor vehicle accident

Figure 8-44. Mechanism of injury of study population

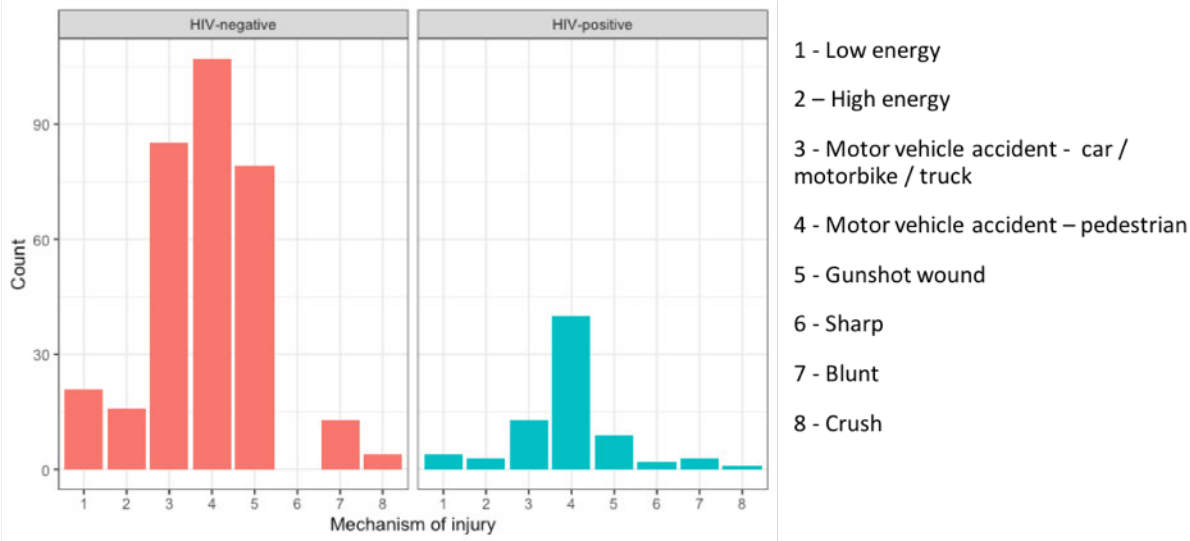
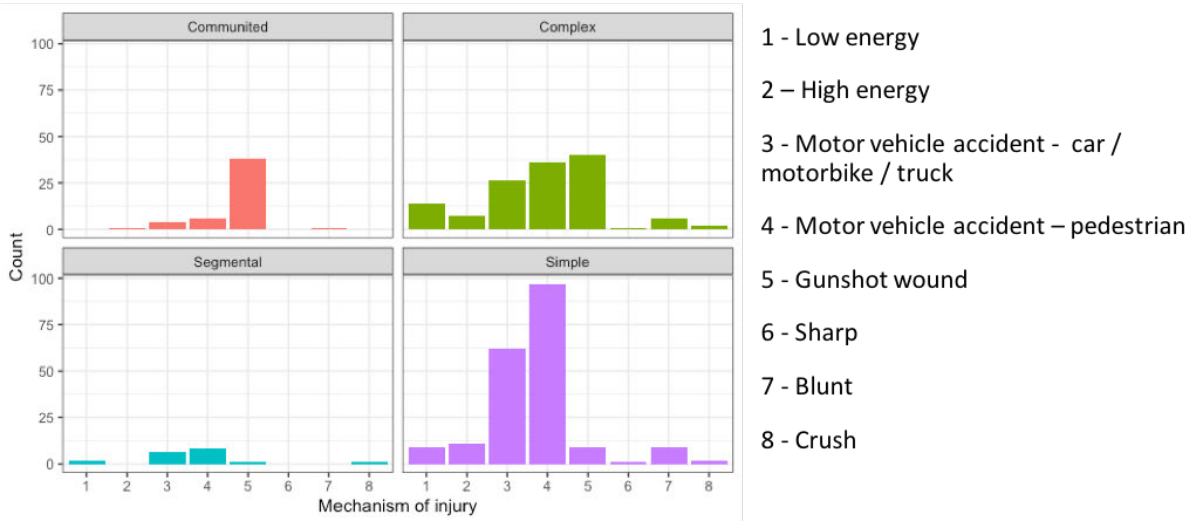


Figure 8-45. The mechanism of injury of study population, stratified according to fracture severity classification



8.5.1.9 Open fracture and additional injuries

There were 161 open fractures (36.4%) in 442 tibia and femur fractures that required IM nailing across the two study sites(Table 8-13). The majority of open fractures were Gustilo Anderson (GA) type I injuries (113/161) (Appendix 13-11). There were 95 GSW fractures and the majority of these resulted in GA type I injuries (96.8%, 92/95). Therefore 92 out of 161 (57.1%) open fractures were due to low velocity GSWs fractures.

More than 97% of participants had their antibiotics within 24 hours of their injury (97.5, 157/161) and all the participants were given antibiotics according to hospital guidelines prior to their surgical procedure. HIV participants waited a longer period of time for their antibiotics following their injury (p=value=0.02). The median length of time between a participant's date and time of injury and the date and time of their surgery was 59.5 (27.15-106.25) / 2.51 (1.13 – 4.42) hours.

A high proportion of participants underwent IM nailing as a single procedure, without an initial washout, or application of external fixator (88.91%, 143/161). At both study sites, all low velocity GSW fractures have their bullet entry and exit wounds left to heal by secondary intention and this reflects the high proportion of participants who had their open fracture wounds left open following their first surgical procedure (67.1%, 108/161) Across both study sites all GSW are then given a short course of antibiotics following their surgery. However, this information was not collected as part of this study protocol.

Due to the nature of the energy required to fracture the tibia or femur, participants commonly had other injuries as well as their fractures (31.3%, 125/400). The Injury Severity Score (ISS) is an established medical score to assess trauma severity.(203), (417) A 'polytrauma' was defined as the ISS being greater than 15.(417) A 'polytrauma' has been shown to be associated with a 5.8-10% increase in mortality.

(418), (419) A full explanation of how the IIS is calculated can be found in Appendix 13-12. Twenty percent (80/400) of the participants had an IIS greater of 16 or great and were therefore classed as suffering from polytrauma.

There was no difference in IIS in HIV-positive compared to negative participants. (p – value = 0.87) When comparing the HIV-negative and positive cohorts, there was a higher proportion of open injuries in the negative group (39.2%, 139/355 vs 25.3%, 22/87) but this was not statistically significant (p-value = 0.87).

Table 8-13 Open fracture parameters of study population

	Study cohort n=442 (%)	HIV-negative n=355 (%)	HIV-positive n=87 (%)	P value
Open fracture				
Yes	161 (36.4)	139 (39.2)	22 (25.29)	0.059
No	281 (63.6)	216 (60.9)	65 (74.71)	
Gustilo Anderson Classification				
I	113 (70.2)	98 (70.5)	15 (68.2)	0.400
II	8 (4.97)	8 (5.8)	0	
IIIA	31 (19.3)	24 (17.3)	7 (31.8)	
IIIB	8 (5.0)	8 (5.8)	0	
IIIC	1 (0.6)	1 (0.6)	0	
Antibiotics commenced post-injury [a] (hrs)				
< 3	10 (6.2)	10 (7.2)	0	0.020
3-6	16 (9.9)	14 (10.3)	2 (9.1)	
6-12	99 (61.5)	89 (64.1)	10 (45.4)	
12-24	32 (19.9)	22 (15.8)	10 (45.5)	
>24	4 (2.5)	4 (2.9)	0	
Open fracture initial management [a]				
IM nailing	143 (88.8)	124 (89.2)	19 (86.4)	0.400
Washout and plaster only	5 (3.1)	5 (3.6)	0	
Washout and external fixator	13 (8.1)	10 (7.3)	3 (13.6)	
Length of time between injury and IM nailing (Median, IQR, hours)	59.5 (27.15-106.25)	55.0 (27.0-99.1)	88.0 (37-140.15)	0.100
Open fracture wounds closure during first procedure [a]				
Yes	53 (32.9)	42 (30.2)	11 (50.0)	0.100
No	108 (67.1)	97 (69.8)	11 (50.0)	

Additional injuries[b]				
Yes	125 (31.3)	99 (30.5)	26 (34.7)	0.401
No	275 (68.7)	226 (69.5)	49 (65.3)	
Injury severity score ≥16 [b]				
Yes	80 (20.0)	64 (19.7)	16 (21.3)	0.873
No	320 (80.0)	261 (80.3)	59 (78.7)	

a: n=161 / 139 /22

b: n=400 / 325 / 75)

IQR – interquartile range

8.5.1.10 Surgical parameters

All 442 fractures underwent reamed locked (proximally and distally) IM nailings across the two study sites (Table 8-14). The procedures were undertaken predominantly by registrar or equivalent training level surgeons (98.4%, 435/442). 99.1% of participants had antibiotics prior to their surgical procedures, according to their hospital policy. Over 50% of procedures were performed out of normal daytime working hours (229/442, 51.8%).

In order to achieve reduction of the fracture for prior to fixation, 18.78% (83/442) of the fractures required the skin to be opened over fracture to gain satisfactory reduction. This proportion was higher in the HIV-positive (26.44%, 23/87) compared to the HIV-negative (18.59% 66/355) participants, but this difference was not statistically significant (p-value=0.828). Two hundred and seventy-seven of the fractures (62.67%) took between one-two hours to complete the surgery, with just 2.05% (9/442) lasting over four hours. Furthermore, (29.64 % (131/442) of participants underwent additional procedures as well as their IM nailing.

Table 8-14. Surgical parameters of study population

	Study cohort n=442 (%)	HIV-negative n=355 (%)	HIV-positive n=87 (%)	p-value
Lead surgeon				
Consultant	6 (1.4)	6 (1.7)	0	0.900
Registrar/fellow	435 (98.4)	348 (98.0)	87 (100.0)	
Intern	1 (0.2)	1 (0.3)	0	
Length of time between injury and surgery (median, IQR, hours)	69.2 (33.6-121.7)	67.5 (30.1-121.1)	70.75 (45.2-126.8)	0.100
Time of day procedure performed				
Morning (0701-1200)	109 (24.7)	85 (23.9)	24 (27.6)	0.621
Afternoon (1201-1700)	104 (23.5)	77 (21.7)	27 (31.0)	
Out of hours (1701-0700)	229 (51.8)	193 (54.4)	36 (41.4)	
Size of nail				
8.5	6 (1.4)	5 (1.4)	1 (1.2)	0.521
10	304 (68.8)	247 (69.6)	57 (65.5)	
11.5	122 (27.6)	98 (27.6)	24 (27.6)	
13	1 (0.2)	1 (0.3)	0 (0)	
Other	9 (2.0)	4 (1.1)	5 (5.8)	
Approach – tibia [a]				
Supra-patellar	167 (77.7)	131 (76.6)	36 (81.8)	0.451
Intra-patellar	48 (22.3)	40 (23.4)	8 (18.2)	
Approach femur [b]				
Antegrade	141 (62.1)	119 (64.7)	22 (51.2)	0.321
Retrograde	86 (37.9)	65 (35.3)	21 (48.8)	
Fracture opened to aid reduction				
Yes	83 (18.8)	66 (19.0)	23 (26.4)	0.828
No	359 (81.2)	289 (81.4)	64 (73.6)	
Duration of procedure (hrs)				
<1	52 (11.8)	39 (11.0)	13 (14.9)	0.843
1-2	277 (62.7)	220 (62.0)	57 (65.5)	
2-4	104 (23.5)	89 (25.1)	15 (17.2)	
>4	9 (2.1)	7 (1.9)	2 (2.3)	

a: n=215 / 171 / 44

b: n=227 / 184 / 43

8.5.1.11 Classification and fracture pattern

In the study population 50.9% (225/442) of the 442 fractures were classified as a simple fracture pattern, according to the AO classification system (Appendix 13-8, Table 8-15). Femur fractures were more likely to be classified as a complex (33.5%, 76/227 vs 20.9%, 45/215) or comminuted (16.3%, 37/227 vs 7.4%, 16/215) fracture pattern when compared to tibia fractures. In both tibia and femur fracture, the HIV-positive participants had a higher proportion of simple fractures, compared to their HIV-negative counter parts (63.2%, 55/87 vs %, 53.8%, 191/355) (Figure 8-46). Therefore, overall there was a higher proportion of unstable and comminuted fractures in the HIV-negative participants (41.4%, 147/355 vs 31.0%, 27/87). Overall, although there was a proportional difference between the two groups, this was not statistically significant (p-value=0.300).

When assessing the femur fracture classification using the Winquist classification (Appendix 13-9), the higher the number on the classification system the more unstable and comminuted the fracture. The HIV-negative participants had a higher percentage of comminuted type III and IV injuries (37%, 68/184 vs 25.6%, 11/43), but again there was no statistical difference between the groups (p-value=0.910).

Comminuted and complex fractures were more likely to result in open fractures and in participants who suffered from polytrauma (Figure 8-48). When classifying the fractures according to the complexity of the injury, the GSW injuries caused a highest proportion of comminuted (39/53, 73.6%) and complex fractures (42/121, 34.7%) amongst the study population(Table 8-16). This suggests that GSW injuries result in more severe and complex fractures. Another important observation was that when hit by a car, pedestrians generally had a higher proportion of complex (31/121, 25.6%), compared to those who sustained injuries sustained whilst in a vehicle (26/121, 21.5%).

Table 8-15. The AO Fracture classification and pattern of study population

	Study cohort n= 442 (%)		HIV-negative n= 355 (%)		HIV-positive n= 87 (%)		p-value
AO Classification							
	Femur[b]	Tibia[c]	Femur[d]	Tibia[e]	Femur[f]	Tibia[g]	0.300
Simple – stable	107	139	82	109	25	30	
A2, A3, B2	(47.1)	(64.7)	(44.6)	(63.7)	(58.1)	(68.2)	
Total	246 (55.6)		191 (53.8)		55 (63.2)		
Complex - unstable	76	45	65	36	11	9	
A1, B1, B3	(33.5)	(20.9)	(35.3)	(21.1)	(25.6)	(20..5)	
Total	121 (27.4)		101 (28.5)		20 (23.0)		
Comminuted - highly unstable C1, C3	37 (16.3)	16 (7.4)	33 (17.9)	13 (7.6)	4 (9.3)	3 (6.8)	
Total	53 (12.0)		46 (13.0)		7 (8.0)		
Segmental - potentially unstable C2	7 (3.1)	15 (7.0)	4 (2.2)	13 (7.6)	3 (7.0)	2 (4.5)	
Total	22 (5.0)		17 (4.8)		5 (5.7)		
Winquist classification							
Femur[a]							
Type 0	42 (18.5)		34 (18.5)		8 (18.6)		0.910
Type 1	62 (27.3)		48 (26.1)		14 (32.6)		
Type 2	44 (19.4)		34 (18.5)		10 (23.3)		
Type 3	57 (25.12)		50 (27.2)		7 (16.3)		
Type 4	22 (9.7)		18 (9.8)		4 (9.3)		

a: n=227

b: n=227

c: n=215

d: n=184

e: n=171

f: n=43

g: n=44

Table 8-16 Fracture classification and mechanism of injury

Mechanism of injury	Simple n=246	Complex n=121	Comminuted n=53	Segmental n=22
Low energy	14 (5.7)	11 (9.1)	0 (0)	3 (13.6)
High energy	13 (5.3)	5 (4.1)	2 (3.8)	0 (0)
MVA: car/motor/bike/truck	79 (32.1)	26 (21.5)	5 (9.4)	7 (31.8)
MVA – pedestrian	112 (45.5)	31 (25.6)	6 (11.3)	10 (45.5)
Gunshot wound	13 (5.3)	42 (34.7)	39 (73.6)	1 (4.5)
Sharp	1 (0.4)	1 (0.8)	0 (0)	0 (0)
Blunt	12 (4.9)	3 (2.5)	1 (1.9)	0 (0)
Crush	2 (0.8)	2 (1.7)	0 (0)	1 (4.5)

MVA: Motor vehicle accident

Figure 8-46. Fracture pattern in HIV-positive and negative participants, according to AO classification system

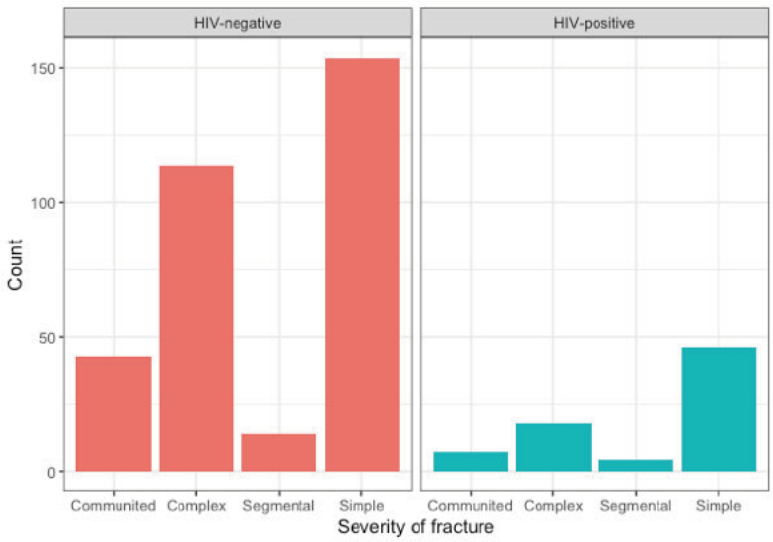


Figure 8-47. Fracture pattern in closed and open fractures, according to AO classification system

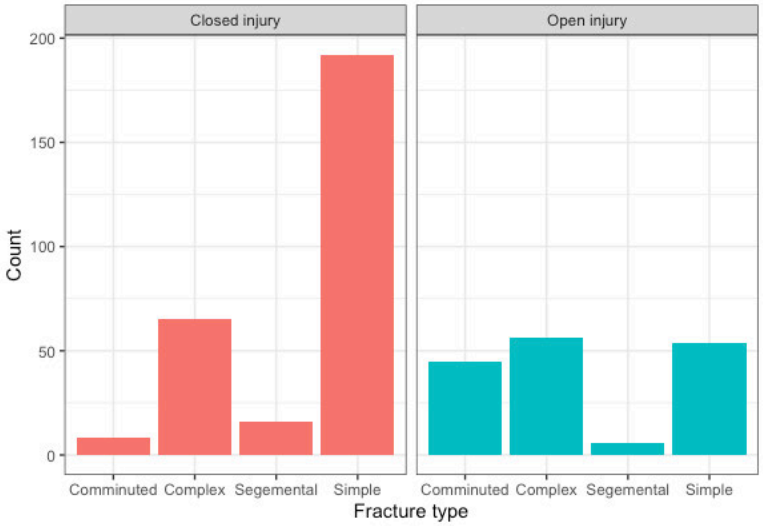
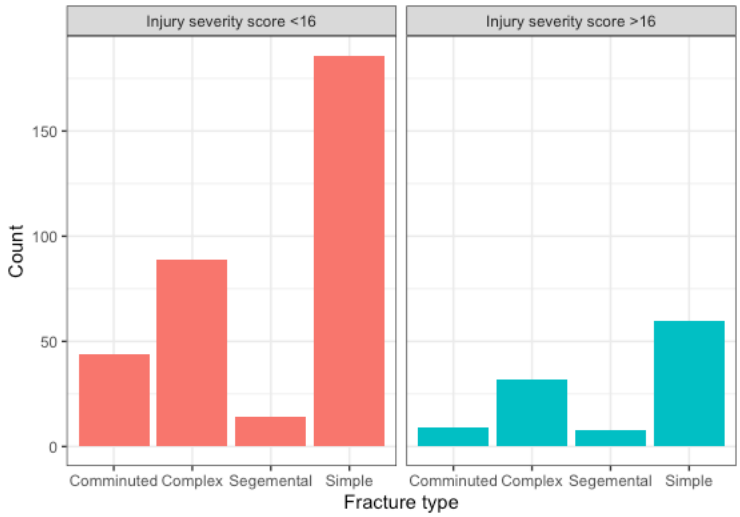


Figure 8-48. Fracture pattern in polytrauma patients (IIS >16)



CHAPTER 9. HIV IN ORTHOPAEDIC SKELETAL TRAUMA 1 STUDY: Analysis of the Primary and Secondary Outcomes and Discussion

9.1 Aims of this chapter

This chapter details the analysis of the primary and secondary outcomes of the HOST 1 Study and ends with a discussion regarding the study.

9.2 Results

9.2.1 Participants lost to follow-up

400 participants, who had undergone 442 IM nailings, were initially recruited to the study. 42 (47 IM nailings) were lost to follow-up before outcome assessment and were excluded from primary analysis. None of the 42 participants had a developed a deep or superficial surgical site infection before being lost to follow-up. Four of the participants (four IM nailings) who were lost to follow-up were HIV-positive. This resulted in a lost to follow-up rate of 10.5%. A summary of the reason for the participants not completing outcome assessment can be seen in Table 9-1.

Table 9-1. Summary of the number of participants lost to follow-up

Reason for lost to follow-up	**Participants n = 400, %**	**IM nailings n =442, %**
Not contactable	34 (8.5)	39 (8.8)
Hardware failure	2 (0.5)	2 (0.5)
Shot dead[a]	6 (1.5)	6 (1.4)
Total	42 (10.5)	47 (10.6)

IM – intra-medullary

a – shot dead after the initial injury from which they were enrolled

On both occasions where a participant had hardware failure following surgery, this was within two weeks of surgery. It was decided by two independent reviewers that

this was an intra-operative technical issue and it did not meet the definition of non-union for this study. Therefore, it was not classified as a non-union.

9.2.2 Final study population

The final study population included 358/400 (89.5%) participants, (Figure 9-1) who underwent 395/442 (89.4%) IM nailings; all participants were followed up for a minimum of 12 months. Of these, 71 participants (71/358, 19.8%) were HIV-positive (83 IM nailings (83/395. 21.0%). A full summary of the baseline characteristics of the final study cohort can be seen in Table 9-2.

Figure 9-1. Flow diagram of final study population

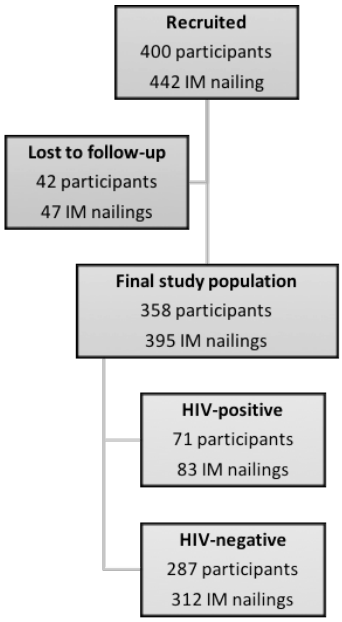


Table 9-2. Summary of baseline characteristics of final study cohort.

Characteristic	Enrolled	Lost to follow up	Final study cohort
Participants	400 (100.0)	42 (10.5)	358 (89.5)
IM nailings	442 (100.0)	47 (10.6)	395 (89.4)
Sex [a]			
Male	313 (78.3)	40 (95.2)	273 (76.2)
Female	87 (21.7)	2 (4.8)	85 (23.7)
Age [a] (yrs: median, IQR)	32.36 (18-71)	30 (20-49)	33 (18-71)
Fracture site [b]			
Tibia	215 (48.6)	19 (40.4)	198 (50.1)
Femur	227 (51.4)	28 (59.6)	197 (49.9)
HIV status [a]			
Positive	75 (18.8)	4 (9.5)	71 (19.8)
Negative	325 (81.2)	38 (90.5)	287 (80.2)
Taking ART on admission [c]			
Yes	42 (56.0)	1 (25.0)	41 (57.7)
No	33 (44.4)	3 (75.0)	30 (42.3)
Crowding index [a]	1.33 (0.25-7)	1.33(0.25-6)	1.33 (0.25-7)
Mechanism of injury [b]			
Low energy	28 (6.3)	1 (2.1)	27 (6.8)
High energy	20 (4.5)	2 (4.3)	18 (4.6)
Motor vehicle accident - car/motorbike/truck	117 (26.5)	9 (19.1)	108 (27.3)
Motor vehicle accident – pedestrian	159 (36.0)	13 (27.7)	146 (37.0)
Gunshot wound	95 (21.5)	20 (42.6)	75 (19.0)
Sharp	2 (0.5)	0	2 (0.5)
Blunt	16 (3.6)	2 (4.3)	14 (3.5)
Crush	5 (1.1)	0	5 (1.3)
Open fracture [b]			
Yes	161 (36.4)	24 (51.1)	137 (34.7)
No	281 (63.6)	23 (48.9)	258 (65.3)
Injury severity score >16 [a]			
Yes	80 (20.0)	7 (16.6)	73 (20.4)
No	320 (80.0)	35 (83.3)	285 (79.6)

IM - intramedullary

IQR – Interquartile range

a – n = 400 / 42 / 358

b – n = 442 / 47 / 395

c – n =75 / 4/ 71

9.2.3 Study outcomes

Delayed union

Overall, 17.4% (69/395) of the study population developed delayed union (Table 9-3). 12 participants with delayed union were HIV-positive (12/395, 3.0%), whereas 57 (57/395, 14.4%) participants with delayed union were HIV-negative. 14.5% of HIV-positive participants developed delayed union (12/83), compared to 18.3% (57/312) in the HIV-negative cohort (univariate odds ratio (OR) 0.76 [95% confidence interval (CI) 0.37-1.44], p-value = 0.417) (Table 9-3).

Non-union

There was a non-union risk of 5.8% (23/395) in the study population with again a slightly higher proportion of non-unions in the HIV-negative cohort (7.1% (22/312) vs 1.2 % (1/83)), compared to the HIV-positive participants (univariate OR 0.16 [CI 0.01-0.78], p-value=0.076).

Infection

The number of deep surgical site infection (DSI) and superficial site infection (SSI) in the overall study population was 5.3% (21/395) and 1.5% (6/395) respectively. The proportion of DSI in the HIV-positive cohort was higher compared to those who were HIV-negative, but not significantly so (8.4% [7/83] vs 4.5% [14/312]), (univariate OR 1.96 [CI 0.72-4.89], p-value=0.161); SSI proportions were very similar (HIV-positive 1.2% [1/83] vs HIV-negative 1.6% [5/312]). A higher proportion of HIV-positive participants presented with late infections (6.0% [5/83] vs 0.6% [2/312]), compared to HIV-negative participants.

Table 9-3. Outcomes of the HOST 1 Study

Outcome	IM Nailings n = 395 , %	HIV-negative n = 312, %	HIV-positive n = 83, %	Univariate odds ratio (95% CI)	p-value
Delayed union					
Yes	69 (17.4)	57 (18.3)	12 (14.5)	0.76 (0.369-1.44)	0.417
No	326 (82.5)	255 (81.7)	71 (85.5)		
Non-union					
Yes	23 (5.8)	22 (7.1)	1 (1.2)	0.16 (0.01-0.78)	0.076
No	372 (94.2)	290 (82.9)	82 (98.2)		
Superficial surgical site infection					
Yes	6 (1.5)	5 (1.6)	1 (1.2)	NA[a]	NA[a]
No	389 (98.5)	307 (98.4)	82 (98.2)		
Deep surgical site infection					
Yes	21 (5.3)	14 (4.5)	7 (8.4)	1.96 (0.72-4.89)	0.161
No	374 (94.7)	298 (95.5)	76 (91.6)		
Late infection					
Yes	7 (1.7)	2 (0.6)	5 (6.0)	NA[a]	NA[a]
No	388 (98.2)	310 (99.4)	78 (94.0)		

CI – Confidence intervals

All CI are adjusted for clustering for multiple fractures per participant

a – univariate analysis not performed due to low numbers

9.2.4 Determining delayed union and non-union using the RUST score – inter-observer reliability

Two independent reviewers (reviewer 1 and reviewer 2), blinded to the HIV status of the participant, reviewed all x-rays. They determined the primary outcome (delayed union) at six-month follow-up or before and the secondary outcome of non-union at nine-month follow-up or before, using the RUST score for both the femur and tibia. On 11 x-rays, the reviewers disagreed on the outcome. Therefore, a third reviewer (reviewer 3) was used to determine consensus. The third reviewer was again blinded to the HIV status of the participant and also the score the two reviewers had given for the x-ray they were reviewing.

On nine occasions the third reviewer was required in order to determine consensus for the primary outcome of union or delayed union at 6 months or before (9/395). The inter-observer agreement, between reviewer 1 and 2, of union or delayed union at this time point determined from the RUST score was 97.7% (Kappa = 0.92).

On two occasions reviewer 3 was needed following a lack of consensus for the secondary outcome of non-union at nine months or before (2/69). The inter-observer agreement, between reviewer 1 and 2, of union or non-union at this time point determined from the RUST score was 97.1% (Kappa = 0.94).

9.2.5 Primary outcome

9.2.5.1 Delayed bone union

The primary outcome of the HOST 1 Study was delayed bone union and was defined as impaired bone healing at six months on RUST score (RUST score < 9). (125), (126), (127), (128) 69/395 (17.4%) fractures in the overall study population exhibited delayed bone healing. Parameters and confounding factors included in the univariate and multivariable regression model included the following (selection described in the methods section of Chapter 8):

1. HIV status
2. Age
3. Sex
4. Smoking status
5. Open fracture
6. Deep surgical site infection
7. Post-operative vitamin D
8. Fracture site

Of the 69/395 (17.5%) delayed union fractures, 3% (12/395) of them occurred among HIV-positive participants, compared to 14.5% (57/395) who were HIV-negative. The proportion of delayed unions in HIV-positive participants as a group was 14.5% (12/83) compared to 18.3% (57/312) in HIV-negatives. Using both univariate (OR 0.76 [CI 0.37-1.44] p-value = 0.417) and multivariable logistic regression analysis (OR 1.06 [CI 0.17-1.01] p -value 0.869), these differences were shown not to be statistically significant (Table 9-4).

Female participants made up 23.7% (85/358) of the study population and were significantly less likely to have delayed union compared to male participants on univariate analysis (OR 0.38, [CI 0.16-0.78], p-value=0.014). However, on multivariable analysis, although delayed union was still less likely in the female

population, this was borderline statistically significant (OR 0.41 [CI 0.17-1.01], p-value=0.053), although confidence intervals were wide.

Fifty-seven percent (39/69) of fractures that developed delayed union occurred following an open fracture, with 28.5% (39/137) of all open fracture developing delayed bone union, compared to 11.6% (30/258) of closed injuries. Open fractures were associated with over three-times increased in the odds of developing delayed bone union compared to closed fractures. (Table 9-4) Both univariate (OR 3.02 [CI 1.78-5.18], p-value 0.001) and multivariable (OR 3.13 [CI 1.74-5.63] p-value = 0.001) logistic regression analysis confirmed that an open fractures was associated with a statistically significant increase in the proportion of delayed unions in the study population.

Regarding a participant's age, the odds of delayed union increased by 3% for every year increase in age at the time of fracture (univariate OR 1.02 [1.0-1.05] p-value 0.050, multivariable OR 1.03 [CI 1.00-1.06] p-value = 0.003) in the overall study population.

There was more than a doubling of the odds of delayed union in fractures of the tibia compared to the femur on both univariate and multivariable logistics regression analysis (OR 2.32 [1.36-4.06] p-value 0.003. OR 2.20 [1.21-3.99] p-value = 0.010).

No other factors examined had any significant impact on the risk of developing delayed bone union in this study cohort (Table 9-4).

Table 9-4. Risk factors for the development of delayed bone healing following an IM nailing for a fracture of the tibia or femur.

	Delayed union n=69, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
HIV status					
HIV-negative	57 (14.4)				
HIV-positive	12 (3.0)	0.76 (0.37-1.44)	0.417	1.06 (0.50-2.22)	0.869
Age					
(IQR: per year)	34 (28-45)	1.02 (1.0-1.05)	0.050	1.03 (1.00-1.06)	0.003
Sex					
Male	61 (15.4)				
Female	8 (2.0)	0.38 (0.16-0.78)	0.014	0.41 (0.17-1.01)	0.053
Smoking status					
Smoker	43 (10.6)	1.56 (0.92-2.68)	0.104	1.62 (0.89-2.96)	0.113
Non-smoker	26 (6.6)				
Open fracture					
Yes	39 (9.9)	3.02 (1.78-5.18)	0.001	3.13 (1.74-5.63)	0.001
No	30 (7.6)				
Deep surgical site infection					
Yes	4 (1.0)	1.12 (0.31-3.14)	0.845	0.97 (0.29-3.32)	0.965
No	65 (16.5)				
Vitamin D[a] (IQR: per 1 nmol/L)	49.4 (32.8-72.4)	1.01 (1.00-1.02)	0.111	1.01 (1.00-1.02)	0.181
Fracture site					
Femur	23 (5.8)	2.32 (1.36-4.06)	0.003	2.20 (1.21-3.99)	0.010
Tibia	46 (11.6)				

CI – Confidence intervals

IQR – Interquartile range

All CI are adjusted for clustering for multiple fractures per participant

a - Vitamin D on enrolment

9.2.5.2 Delayed bone union in HIV-positive participants

Seventy-one HIV-positive participants who underwent 83 IM nailings (83/395, 21.0%) reached a primary outcome union or delayed union at six-month follow-up or before. The median CD4 count and viral load were very similar at six-months (413 cells/mm^3, IQR 302-653/2.13 log10 cps/ml IQR 1.3-4.62), compared to baseline (402 cells/mm^3, IQR 265-630/1.99 log10 cps/ml, IQR 1.30-410). There was an 11.3% (8/71) percentage point increase in the number of participants taking ART at six-month follow-up (49/71, 69.0%), compared to baseline (41/71, 57.7%). Those participants who started ART after enrollment commenced their therapy within 6 weeks of their study outcome. It is important to highlight that during the study the aim was to have 100% of participants on ART by six months, but due to lack of engagement by the participants this was not achieved A summary of these parameters can be found in Table 9-5.

Table 9-5.Summary of HIV participant parameters at baseline and 6-month follow-up

	Baseline **n = 71, %**	**6 months** **n = 71, %**
CD4 (IQR, (cell/mm3)	402 (264-630)	413 (302-653)
Viral load (IQR: Log10, median, cps/ml)	2.13 (1.3-4.62)	1.99 (1.30-4.10)
Taking ART		
Yes	41 (57.7)	49 (69.0)
No	30 (42.3)	22 (31.0)

ART – Anti-retroviral therapy

IQR – Interquartile range

As discussed, 12/83 (14.5%) fractures in HIV-positive participants developed delayed bone union following their injuries. This small number of participants makes it difficult to draw valid conclusions. The proportion of delayed union in ART naïve participants was 23.3% (7/30), compared to 12.2% (5/41) in those participants taking ART on enrolment.

The following parameters and confounding factors were included in the univariate and multivariable logistic regression models to analysis delayed union in the HIV-positive study population:

1. Age
2. Sex
3. Open fracture
4. Viral load
5. CD4 count
6. ART treatment

Two different univariate and multivariable logistic regression models were constructed. One included CD4 count, viral load and ART treatment at baseline (Model 1, Table 9-6) and one included CD4 count, viral load and ART treatment at six-months follow-up. (Model 2, Table 9-7)

In the two models, following both univariate and multivariable logistic regression analysis, none of the parameters included were shown to be statistically significant for the development of delayed bone union within the HIV-positive population. A full summary of the univariate and multivariable logistic regression analysis can be seen in Table 9-6 and Table 9-7

The median CD4 count at baseline in the delayed union participants were similar at 460 cell/mm^3 (IQR 366 -477) compared to 413 cell/mm^3 (IQR 295-673, [p-value - 0.4]) in healed participants. (Figure 9-2, Table 9-8) The median viral load in the delayed

union participants was higher (3.02 [IQR 0.98-4.76]) compared to (2.13 [IQR 1.30-4.40]) [p-value = 0.001]) in the healed participants.

Table 9-6. HIV-positive study population baseline: Risk factors for the development of delayed bone healing following an IM nailing for a fracture of the tibia or femur.

	Delayed union n=12, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
Age (IQR, per year)	34.5 (30.5-39.8)	1.01 (0.95-1.08)	0.740	1.00 (0.90-1.12)	0.960
Sex					
Male	11 (13.3)				
Female	1 (1.0)	0.13 (0.01-0.73)	0.059	0.254 (0.01-1.84)	0.236
Open fracture					
Yes	6 (7.2)	3.44 (0.96-12.5)	0.055	2.93 (0.57-15.2)	0.189
No	6 (7.2)				
CD4 count on admission (IQR:cell/mm3)	540 (352-618)	1.00 (0.97-1.00)	0.956	1.00 (0.96-1.00)	0.830
Viral load on admission (IQR: log10, cps/ml)	3.02 (0.976-4.26)	1.09 (0.73-1.63)	0.672	1.02 (0.65-1.63)	0.940
Taking ART on admission					
Yes	5 (6.0)	0.47 (0.13-1.60)	0.227	1.16 (0.17-8.77)	0.189
No	7 (8.4)				

ART – anti-retroviral therapy

CI – confidence intervals

IQR – interquartile range

All CI are adjusted for clustering for multiple fractures per participant

Table 9-7. HIV-positive study population 6-month follow-up: Risk factors for the development of delayed bone healing following an IM nailing for a fracture of the tibia or femur.

	Delayed union n=12, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
Age (IQR: per year)	34.5 (30.5-39.8)	1.01 (0.95-1.08)	0.740	1.06 (0.97-1.18)	0.194
Sex					
Male	11 (13.3)				
Female	1 (1.0)	0.13 (0.01-0.73)	0.059	0.23 (0.01-1.56)	0.194
Open fracture					
Yes	6 (7.2)	3.44 (0.96-12.50)	0.055	2.27 (0.44-11.4)	0.311
No	6 (7.2)				
CD4 count at six months (IQR:Cell/mm3)	406 (366-477)	1.00 (0.98-1.00)	0.322	1.00 (0.97-1.00)	0.181
Viral load at six months (IQR: cps/ml)	3.02 (0.976-4.76)	1.13 (0.78-1.63)	0.522	1.03 (0.66-1.58)	0.897
Taking ART at six months					
Yes	6 (7.2)	0.31 (0.87-1.13)	0.071	0.45 (0.08-2.41)	0.336
No	6 (7.2)				

ART – anti-retroviral therapy

CI – confidence intervals

IQR – interquartile range

All CI are adjusted for clustering for multiple fractures per participant

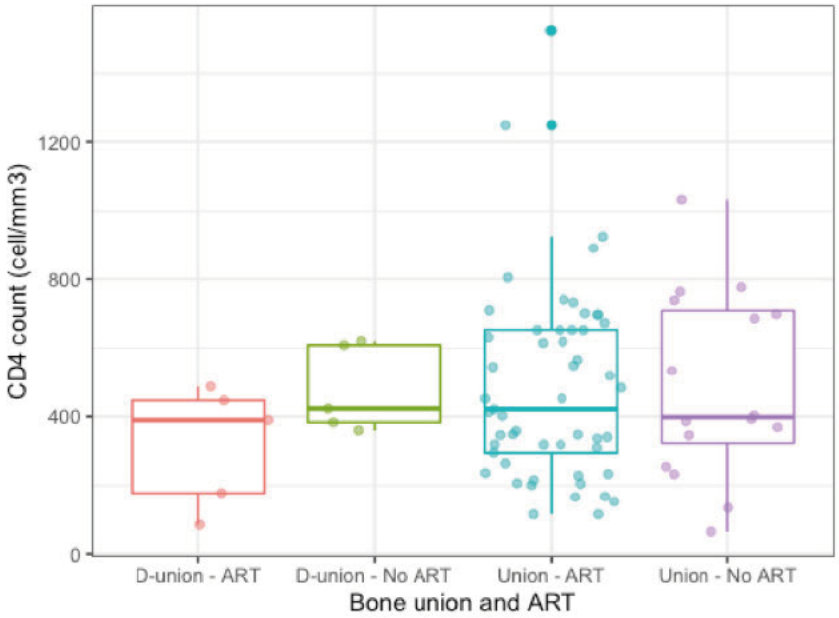
Table 9-8. The CD4 count of participants according to the participants primary outcome and ART therapy

	Baseline			
	Union – ART n = 54	**Union – No ART n = 17**	**Delayed - ART n = 6**	**Delayed union no ART n = 6**
CD4 count cell mm3 (Median: IQR)	421 (295-653)	398 (323-709)	390 (178-447)	423 (383-609)

IQR – interquartile range

Means have been reported due to the low number of participants in the non-union groups.

Figure 9-2. A box and whisker plot summary of the CD4 count according to the primary outcome of delayed union and if the participant was taking anti-retroviral therapy. (note: D-union = delayed bone union)



9.2.6 Secondary outcome

9.2.6.1 Non-union

Fracture non-union was defined as one or both of the following:

- Need for further surgery to achieve union before nine months. This decision was made by two orthopaedic surgeons. (125), (126), (127), (128).
- Impaired bone healing at nine months on RUST score (RUST score < 9). (125), (126), (127), (128) *Note all non-unions were diagnosed with this definition.*

There were 23/395 (5.8%) fractures that developed non-union in the study population.

Parameters and confounding factors included in the univariate and multivariable regression model included the following:

1. HIV status
2. Age
3. Sex
4. Smoking status
5. Open fracture
6. Post-operative haemoglobin
7. Post-operative vitamin D

Deep surgical site infection was removed from this model since only one participant, who was HIV-negative had both deep surgical site infection and non-union. Fracture site was not included since a similar number of femur and tibias developed non-union and this did not add any improvement in fit to the models (femur 11/23 [47.8%], tibia 12/23 [52.2%]).

Of the 23/395 (5.8%) non-unions, 0.3% (1/395) were in HIV-positive participants, compared to 5.6% (22/395) who were HIV-negative. There was a higher proportion

of non-union in the HIV-negative cohort (7.1% [22/312] vs 1.2 % [1/83]), compared to the HIV-positive participants. On both univariate logistic (OR 0.16 [CI 0.01-0.78]) and multivariable regression (OR 0.17 [CI 0.01-0.92]) models the associations were statistically significant.

Sex did not have a statistically significant effect on non-union on univariate analysis, although odds of non-union were 70% higher in men compared to women (OR 0.30 [CI 0.05-1.04] p-value = 0.107). On multivariable analysis estimates were less precise and non-significant (OR 0.51 [CI 0.07-2.01], p-value = 0.400).

65% (15/23) of all the non-unions resulted following an open fracture. 10.9% (15/137) of all open fractures developed a non-union in the study population, compared to 3.1% (8/258) of closed fractures. Non-union was nearly four times more likely following an open fracture in univariate (OR 3.84 [CI 1.62-9.77] p-value = 0.003) and nearly three times as likely in multivariable analysis (2.96 [CI 1.16-8.07] p-value = 0.026) respectively.

On multivariable logistic regression analysis, the odds of non-union increased by 3% for every 1 nmol/L increase in vitamin D at baseline enrolment (multivariable OR 1.03 [CI 1.01-1.05] p-value = 0.009).

Table 9-9. Risk factors for the development of non-union following an IM nailing for a fracture of the tibia or femur in this study population

	Non union n=23, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
HIV status					
HIV-negative	22 (5.6)				
HIV-positive	1 (0.3)	0.16 (0.01-0.78)	0.076	0.17 (0.01-0.92)	0.100
Age (IQR: per year)	36 (28-48.5)	1.00 (0.96-1.04)	0.935	1.03 (0.99-1.07)	0.215
Sex					
Male	21 (5.3)				
Female	2 (0.5)	0.30 (0.05-1.04)	0.107	0.51 (0.07-2.01)	0.400
Smoking status					
Yes	16 (4.1)	1.95 (0.80-5.19)	0.116	1.67 (0.68-4.67)	0.303
No	7 (1.8)				
Open fracture					
Yes	15 (3.8)	3.84 (1.62-9.77)	0.003	2.96 (1.16-8.07)	0.026
No	8 (2.0)				
Haemoglobin [a] (IQR: per 1 g/dL)	10 (7.72-13.0)	0.93 (0.78-1.11)	0.423	0.92 (0.75-1.11)	0.395
Vitamin D [a] (IQR: per 1 nmol/L)	55.9 (40.3-79.6)	1.02 (1.00-1.03)	0.08	1.03 (1.01-1.05)	0.009

CI – Confidence interval

IQR – interquartile range

a – Measured on enrolment

All CI are adjusted for clustering for multiple fractures per participant

9.2.6.2 Deep surgical site infection

Deep surgical site infection was defined using The Centre for Disease Control and Prevention definition of a wound infection involving the tissues deep to the skin that occurs within 30 days of injury (closed reduction of fracture) or 90 days (open reduction of fracture) (where day 1 = the procedure date.) (363)

There were 21/395 (5.3%) fractures that developed DSI in the study population. Parameters and confounding factors included in the univariate and multivariable regression model included the following:

1. HIV status
2. Age
3. Sex
4. Smoking status
5. Open fracture
6. Post-operative white blood cells

Of the 395 cases, 21 developed DSIs (5.3%), seven (1.8%) of which were in HIV-positive participants. This is compared to 3.5% (14/395) who were HIV-negative. The proportion of DSI in HIV-positive participants was 8.4% (7/83), compared to 4.5% (14/312) in HIV-negative participants. HIV status was not significantly associated with for DSI following IM nailing in the univariate model (OR 1.96 [CI 0.72-4.89] p-value = 0.161) or in the multivariable model (OR 2.59 [CI 0.86-7.80] p-value=0.090) (Table 9-10). The proportion of participants taking ART was similar, with three out of the seven (42.9%) HIV-positive participants taking ART and four not (57.1%). Due to the low numbers, no univariate or multivariable analysis was undertaken.

In those HIV-positive participants who developed DSI, there was no statistically significant difference in their CD4 count at baseline (median CD4 328 Cell/mm3 [200-

822]) compared to participants who did not develop an infection (median CD4 417 Cell/mm3 [306-653]) (OR 1.00 [CI 0.99-1.00] p-value = 0.394).

Open fractures resulted in a higher proportion of participants experiencing DSI. Fifty-seven percent (12/21) of DSI were in participants whose injuries were following an open fracture. This resulted in 8.8% (12/137) of all open fractures developing a DSI, compared to 3.5% (30/258) in closed injuries (univariate OR 2.66 [CI 1.10-6.67], p-value = 0.032, multivariable (OR 3.39 [CI 1.20-9.61] p-value = 0.026).

Males had nearly three times the odds of developing a DSI compared to females, although on univariate and multivariable logistic regression analysis this difference was of borderline statistical significance (univariate OR 1.70 [CI 0.63-4.23] p-value = 0.268, multivariable OR 2.97 [0.97-9.04] p-value = 0.056).

Table 9-10. Risk factors for the development of deep surgical site infection following an IM nailing for a fracture of the tibia or femur in this study population.

	Deep surgical site infection n=21, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
HIV status					
HIV-negative	14 (3.5)				
HIV-positive	7 (1.8)	1.96 (0.72-4.89)	0.161	2.59 (0.86-7.80)	0.090
Age (IQR:per year)	29 (22-42)	0.97 (0.93-1.01)	0.195	0.98 (0.94-1.03)	0.469
Sex					
Male	14 (3.5)				
Female	7 (1.8)	1.70 (0.63-4.23)	0.268	2.97 (0.97-9.04)	0.056
Smoking status					
Yes	11 (2.8)	0.78 (0.32-1.90)	0.585	0.74 (0.26-2.14)	0.578
No	10 (2.5)				
Open fracture					
Yes	12 (3.0)	2.66 (1.10-6.67)	0.032	3.39 (1.20-9.61)	0.026
No	9 (2.3)				
White blood cells [a] (IQR, x 10^9/L)	8.63 (7.35-12.1)	1.04 (0.95-1.12)	0.326	1.06 (0.97-1.16)	0.220

CI – confidence interval

IQR – interquartile range

a – Measured on enrolment

All CI are adjusted for clustering for multiple fractures per participant

9.2.6.3 Superficial surgical site infection

Superficial surgical site infection was defined using the Centre for Disease Control and Prevention definition of a wound infection involving the skin and subcutaneous tissue that occurs within 30 days of surgery (where day 1 = the procedure date).(402)

Only 6/395 (1.5%) fractures developed an SSI in the study population. Due to the low number of SSI, univariate and multivariable logistic regression analysis was not undertaken. Of the 6/395 (1.5%) SSIs, 0.3% (1/395) were in HIV-positive participants, compared to 1.3% (5/395) who were HIV-negative. The proportion of SSI in HIV-positive participants was 1.2% (1/83), compared to 1.6% (5/312) in HIV-negative participants. As with DSI, open fractures resulted in a high proportion of the SSI recorded. Eighty-three percent of SSI (5/6) were in participants whose injuries were following an open fracture (Table 9-11).

Table 9-11. Summary of superficial surgical site infection following an IM nailing for a fracture of the tibia or femur in our study population

	Superficial surgical site infection n=6, %
HIV status	
HIV-negative	5 (1.3)
HIV-positive	1 (0.3)
Age	32 (29-39.5)
(IQR, per year)	
Sex	
Male	6 (1.5)
Female	0 (0)
Smoking status	
Yes	4 (1.0)
No	2 (0.5)
Open fracture	
Yes	5 (1.3)
No	1 (0.3)
White blood cells	
(IQR: x 10^9/L) [a]	8.41 (5.62-8.58)

IQR – interquartile range

a – Measured on enrolment

9.2.6.4 Late implant infection

Late implant infection was defined as any late wound breakdown (>30 days for closed reduction of fractures or >90 days for openly reduced fractures) or sinus formation, or unexplained late pain with associated radiological changes consistent with peri-implant sepsis.(388)

There were 7/395 (1.8%) fractures that developed late implant infection in the study population. Due to the low number of late infections, a univariate and multivariable logistic regression analysis was not undertaken. Of the 7/395 (1.8%) late infections, 1.3% (5/395) were in HIV-positive participants, compared to 0.5% (2/395) who were HIV-negative. The proportion of late infection in HIV-positive participants was 6.0% (5/83), compared to 0.6% (2/312) in HIV-negative participants (Table 9-12). All of the late infections were in participants who had fractures that had healed.

Table 9-12. Summary of late implant infection following an IM nailing for a fracture of the tibia or femur in our study population

	Late infection n=7, %
HIV status	
HIV-negative	2 (0.5)
HIV-positive	5 (1.3)
Age (IQR: per year)	33 (28.5-38.5)
Sex	
Male	5 (1.3)
Female	2 (0.5)
Smoking status	
Yes	6 (1.5)
No	1 (0.3)
Open fracture	
Yes	3 (0.8)
No	4 (1.01)
White blood cells [a] (IQR, x 10^9/L)	9.3 (7.90-10.5)

IQR – interquartile range

a – Measured on enrolment

9.2.6.5 The length of time a participant diagnosed with HIV and taking anti-retroviral therapy: How this impacted the study outcomes

Participants had the date they first received a positive laboratory blood sample for HIV and the date of their IM nailing surgery recorded. Therefore, it was possible to determine the length of time a participant had been diagnosed with HIV. Furthermore, the length of time a participant was taking ART was recorded.

The interquartile ranges for length of time since diagnosis of HIV and duration of ART were very large, and the number of participants who were HIV-positive and developed delayed union or DSI were low. Therefore, no further statistical analysis and regression modelling was undertaken.

With the limited data available, participants who have had a diagnosis of HIV for longer were more likely to develop delayed union (union: 116.4 month [IQR 61.5-290.8] vs delayed union: 178.2 months [IQR 44.9-324.3]). DSI was more likely in those participants who had been diagnosed for a shorter period of time (DSI 178.2 month [IQR 44.9-323.3] vs no DSI 301.8 months [IQR 163.5-347.0]). Regarding the length of time a participant had been taking ART, the longer the period of time on ART, the more likely a participant was to develop delayed union. The shorter the period of time on ART, the more likely a participant was to developed a DSI (DSI 138.2 months [IQR 57.2-290.8] vs no DSI 301.8 months [IQR 163-331.8]) (Table 9-13, Table 9-14, Figure 9-3). However, due to the low of participants who developed each outcome, no definitive conclusion can be drawn from these results.

Table 9-13. A summary of the how long a participant had a diagnosis of HIV and how this influenced the outcome of delayed union and deep surgical site infection.

	Union n = 71	**Delayed union n = 12**
Length of since HIV diagnosis (IQR: months, median)	116.4 (61.5-290.8)	178.2 (44.9-324.3)
	No deep surgical site infection n = 76	**Deep surgical site infection n = 7**
Length of since HIV diagnosis (IRQ: months, median)	301.8 (163.5-347)	178.2 (44.9-324.3)

CI – confidence interval

IQR – interquartile range

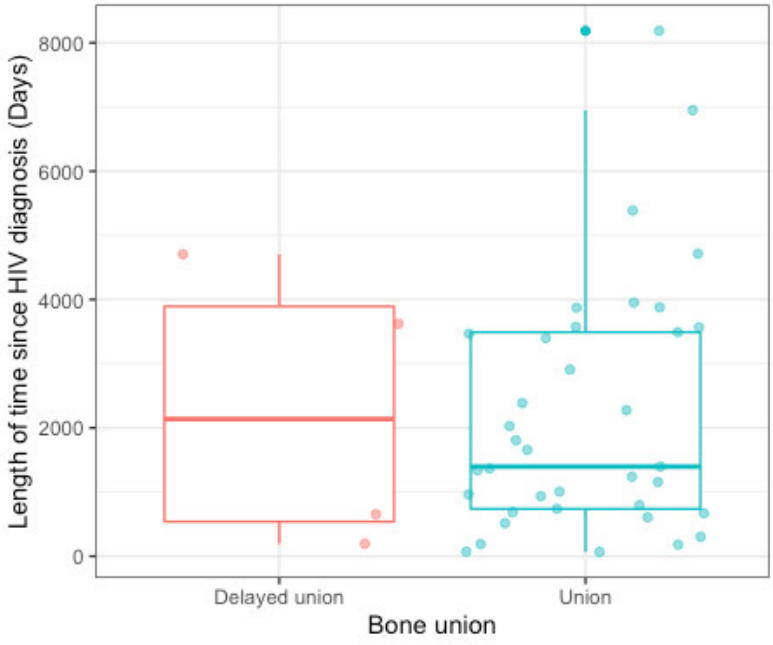
Table 9-14 Summary of how long a participant had been on ART on enrolment and how this influenced the outcome of delayed union and deep surgical site infection.

	Union n = 43	**Delayed union n = 5**
Length of time on ART (IQR: months, median)	144.3 (60.3-292.4)	178.1 (44.1-316.8)
	No deep surgical site infection n = 45	**Deep surgical site infection n = 3**
Length of on ART (IQR: months, median)	301.8 (163.5-331.8)	138.2 (57.2-290.8)

CI – confidence interval

IQR – interquartile range

Figure 9-3. A box and whisker plot of the length of time a participant had been living with HIV and their primary outcome.



9.2.6.6 Open fractures

There were 137/395 (34.7%) IM nailings in 358 study participants with open fractures. Of these 137 open fractures, 22 (16.1%) were in HIV-positive participants. Following an open fracture, HIV does not appear to be a statistically significant factor for the development of delayed union, non-union or DSI and SSI. (Table 9-15) There were a low number of late implants infections in the study cohort (7/395, 1.7%), three of which were in open fracture. Although, on univariate logistic regression analysis the association between HIV and late implant infection was borderline significant (OR 11.40 [1.05-25.20] p-value = 0.051), caution should be made when interpreting this result due to the low numbers.

The proportion of participants who developed delayed union was similar in HIV positive (27.3%, 6/22) and in HIV negative (28.7%, 33/115) participants. When reporting proportional comparisons between non-union, SSI, DSI and late implant infection according to HIV status in open fractures, due to the low number of participants who developed these outcomes overall, it is difficult to draw any valid conclusions from this data. However, the proportion of HIV-positive participants who developed nonunion (0% 0/22 vs 13.0% 15/115) were lower in HIV positive participants. Whereas the proportion of DSI (13.6% 3/22 vs 7.8% 9/115), SSI (4.5% 1/22 vs 3.5% 4/115) late implant infection (9.1% 2/22 vs 0.9% 1/115) were higher in HIV-positive participants compared to HIV-negative.

There were a high number of GA grade I open fractures, due to the high number of GSW fractures included in the study. After excluding all the GA grade I fractures from the analysis there were 44 open fractures GA grade II or above. There were 7/44 open fractures that were HIV-positive with a GA grade of II or above. HIV still did not appear to be a significant risk factor for the development of delayed union (OR 0.59 [CI 0.08-3.13] p-value = 0.554), non-union (0 HIV+ve participants), DSI (OR 2.07 [CI 0.26-12.50]

p-value = 0.444), SSI (0 HIV+ve participants) or late implant infection (0 HIV+ve participants) in open GA grade II plus open fractures. (Table 9-16).

Table 9-15. A summary of the primary and secondary study outcomes in open fractures, assessing HIV as a risk factor

	Delayed union n = 39, %	**Univariate odds ratio (95% CI)**	**p-value**
HIV-positive[a]			
Yes	6 (4.4)	0.93 (0.31-2.49)	0.89
No	33 (24.1)		
	Non-union n = 15		
HIV-positive[a]			
Yes	0 (0)	NA[b]	NA[b]
No	15 (10.9)		
	Deep surgical site infection n = 12		
HIV-positive[a]			
Yes	3 (2.2)	1.86 (0.39-6.91)	0.38
No	9 (6.6)		
	Superficial surgical site infection n = 5		
HIV-positive[a]			
Yes	1 (0.7)	1.32 (0.66-9.50)	0.807
No	4 (2.9)		
	Late implant infection n = 3		
HIV-positive [a]			
Yes	2 (1.5)	11.40 (1.05-25.20)	0.051
No	1 (0.7)		

CI - confidence interval

a = 44

b = not performed due to low number of values

All CI are adjusted for clustering for multiple fractures per participant

Table 9-16. A summary of the primary and secondary study outcomes in open fractures with a Gustilo Anderson grade II or higher, assessing HIV as a risk factor

	Delayed union n = 17, %	**Univariate odds ratio (95% CI)**	**p-value**
HIV-positive[a]			
Yes	2 (4.5)	0.59 (0.08-3.13)	0.554
No	15 (34.1)		
	Non-union n = 8		
HIV-positive[a]			
Yes	0 (0)	NA[b]	NA[b]
No	8 (18.2)		
	Deep surgical site infection n = 8		
HIV-positive[a]			
Yes	2 (4.5)	2.07 (0.26-12.5)	0.444
No	6 (13.6)		
	Superficial surgical site infection n = 3		
HIV-positive[a]			
Yes	0 (0)	NA[b]	NA[b]
No	3 (6.8)		
	Late implant infection n = 0		
HIV-positive[a]			
Yes	0 (0)	NA[b]	NA[b]
No	0 (0)		

CI - confidence interval

a = 44

b = not performed due to low number of values

All CI are adjusted for clustering for multiple fractures per participant

The majority of these fractures were GA type I fracture (93/137, 67.9%), which corelates with the high number of GSW (75/137, 54.7%) injuries included in the study. 41.4% (12/29) of GA type IIIA developed delayed union rate, compared to 23.7% (22/93) of GA type I injuries. Furthermore, the proportion of non-unions (17.2%, 5/29 vs 7.5%, 7/93) and DSIs (17.2, 5/29 vs 4.3%, 4/93) were also higher in the GA type IIIA compared to GA type I. Due to the low number in the GA type II and IIB groups it is difficult to draw valid conclusions but despite this it does appear that as the severity of the GA fracture classification gets worse, so did the proportion of delayed union, non-union and DSI (Table 9-17, Figure 9-4, Figure 9-5).

Table 9-17. Summary of the study outcomes according to Gustilo Anderson open fracture classification.

	GA I n = 93, %	**GA II n = 7, %**	**GA IIIA n = 29, %**	**GA IIB n = 8, %**	**Total n = 137**
Delayed union	22 (23.7)	2 (28.6)	12 (41.4)	3 (37.5)	39 (28.5)
Non-union	7 (7.5)	1 (14.9)	5 (17.2)	2 (25.0)	15 (10.9)
Deep surgical site infection	4 (4.3)	0	5 (17.2)	3 (37.5)	12 (8.8)
Superficial surgical site infection	2 (2.2)	2 (28.6)	1 (3.4)	0 (0)	5 (3.6)
Late infection	3 (3.2)	0	0 (0)	0 (0)	3 (2.2)

GA – Gustilo Anderson

Figure 9-4. Summary of delayed fracture healing according to Gustilo Anderson open fracture grade

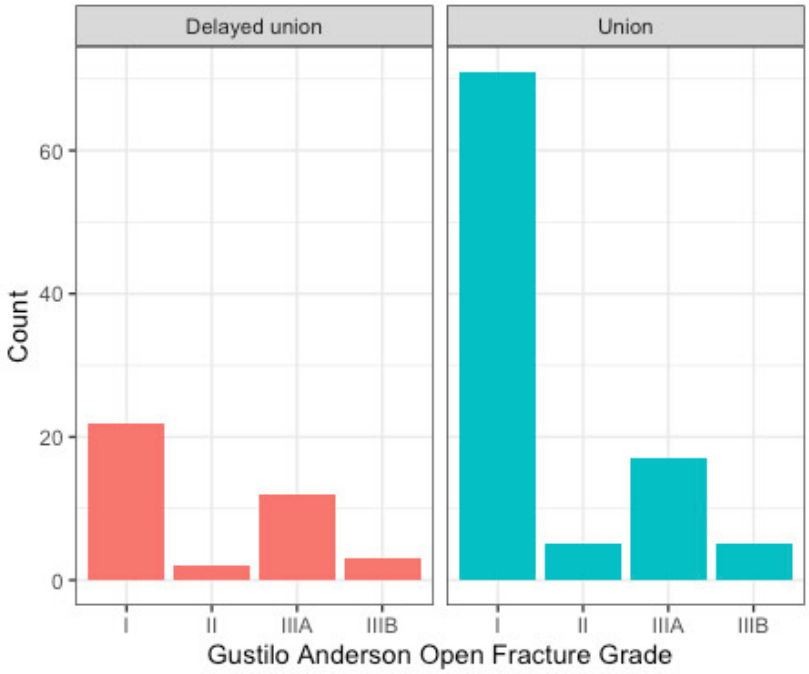
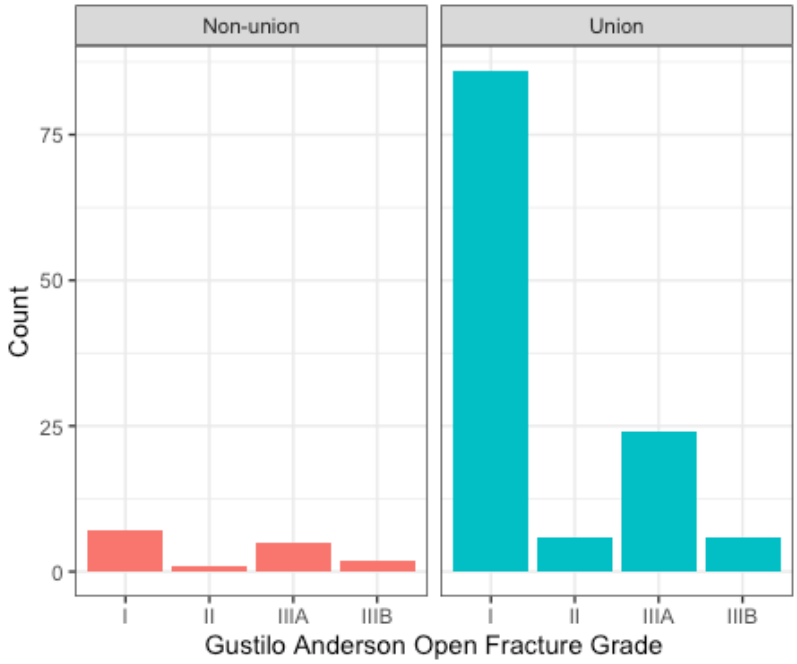


Figure 9-5. Summary of non-union according to Gustilo Anderson open fracture grade



9.2.6.7 Injury severity score

The Injury Severity Score (ISS) is an established medical score to assess trauma severity(203),(417) and has been used to define the term polytrauma. A polytrauma was defined as the ISS being greater than 15 in this study.(417)

In the final study population, 100 (100/395, 25%) participants had an ISS ≥ 16. Out of 83 IM nailings in HIV-positive participants, 22 (26.5%) were in participants with an ISS ≥ 16, compared to 61 (61/312) 19.5% who were HIV-negative.

An ISS ≥ 16 (polytrauma) was not a statistically significant factor for the development of delayed union, non-union, DSI, SSI or late infection on univariate analysis (Table 9-18, Figure 9-6, Figure 9-7). Regarding late implant infection, although the number of participants who developed late implant infection were was low (7/395), 57.1% (4/7) of them were in participants who had sustained a polytrauma (ISS>16), although this was shown not to be statistically significant.

Table 9-18. A summary of the relationship between the Injury Severity Score and primary and secondary outcome in our study population

	Delayed union n = 69, %	**Univariate odds ratio (95% CI)**	**p-value**
ISS ≥ 16			
Yes	19 (4.8)	1.15 (0.62-2.04)	0.641
No	50 (12.7)		
	Non-union n = 23		
ISS ≥ 16			
Yes	5 (1.3)	0.81 (0.26-2.09)	0.685
No	18 (4.6)		
	Deep surgical site infection n = 21		
ISS ≥ 16			
Yes	6 (1.5)	1.19 (0.42-3.02)	0.725
No	15 (3.8)		
	Superficial surgical site infection n = 1		
ISS ≥ 16			
Yes	1 (0.3)	0.59 (0.03-3.69)	0.627
No	5 (1.3)		
	Late implant infection n = 7		
ISS ≥ 16			
Yes	4 (1.0)	4.06 (0.88-20.9)	0.07
No	3 (0.8)		

CI - confidence interval

a = 44

All CI are adjusted for clustering for multiple fractures per participant

Figure 9-6. Summary of the number of participants who developed delayed bone union of a fracture stratified according to their injury severity score on admission

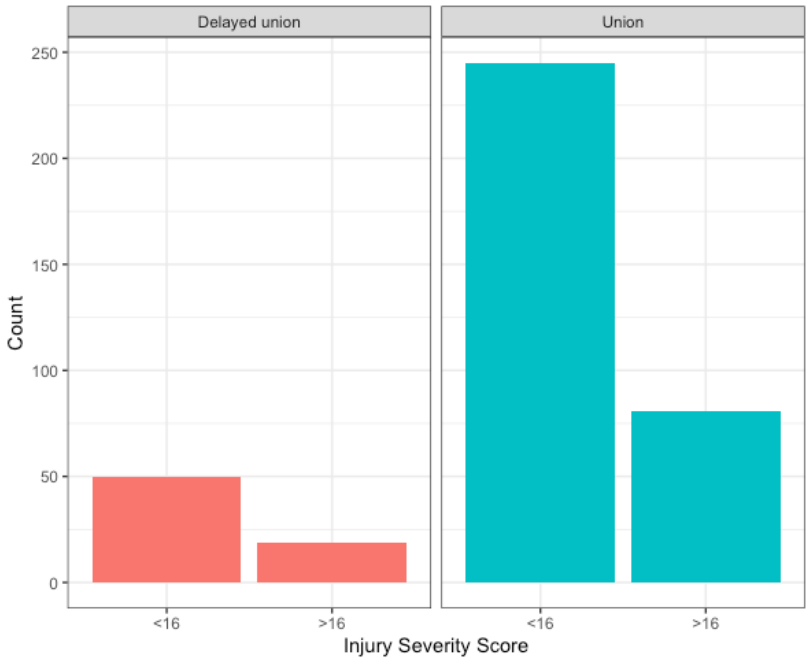
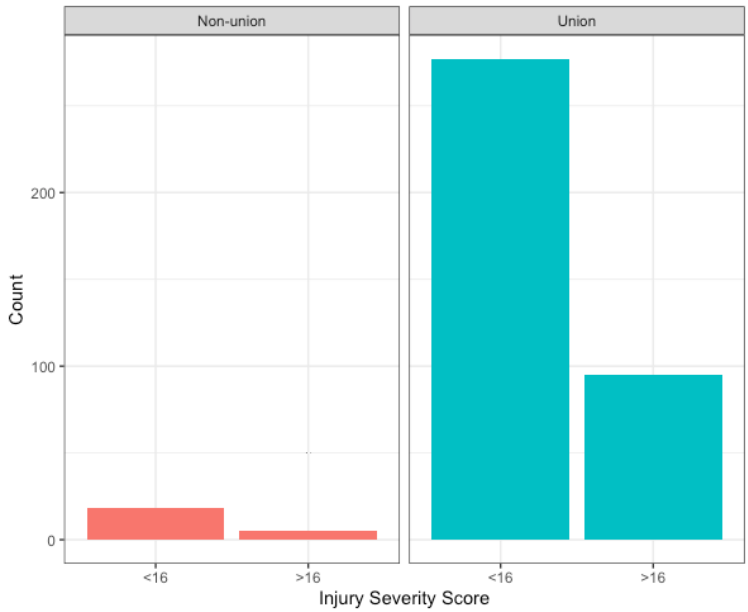


Figure 9-7. Summary of the number of participants who developed non-union of a fracture stratified according to their injury severity score on admission



9.2.6.8 Disability rated index

The median DRI in the study population at baseline, prior to injury, was 0 (IQR 0-34.3) and the DRI at six months was 20 (IQR – 8-33, p value = 0.001). The was no difference in the DRI between the HIV-positive or HIV-negative participants (baseline HIV +ve 0 [IQR 0-33] vs HIV -ve 0 [IQR 0-28], 6 month HIV +ve 19 [IQR 10-29] vs HIV -ve 20 [IQR 8-33]). It is expected that those participants who developed delayed union and non-union following their injuries would have worse functional outcome, hence higher DRI, when compared to those participants whose fracture healed. This was confirmed by the study participants' six-month DRI. There was a higher DRI in participants who developed delayed (median 31 [IQR 14-44] vs median 16.5 [IQR 7-30] and non-union (median 33 [IQR 20-47] VS median 18 [IQR 8-33]). A summary of these findings can be found in Table 9-19.

Table 9-19. The relationship between the disability rated index and primary and secondary outcome in our study population.

	DRI at six-month follow-up n - 358
Delayed healing	
(median: IQR)	
Yes	31 (14-44)
No	16.5 (7-30)
Nonunion	
(median: IQR)	
Yes	33 (20-47)
No	18 (8-33)
Deep Surgical Site Infection	
(median: IQR)	
Yes	20 (12-36)
No	20 (8-32)
Superficial Surgical Site Infection	
(median: IQR)	
Yes	18 (7-24
No	20 (8-33)
Late infection	
(median: IQR)	
Yes	5 (0.75-10.2)
No	20 (8-33)

CI -Confidence interval

IQR -interquartile range

DRI – Disability rated index

9.2.6.9 Waiting time between injury and date of surgery: How this impacted the study outcomes.

The median time between a participant's injury and their surgery was 69.2 hours (33.6-121.7). The time between injury and the date of surgery had no impact on any of the primary or secondary outcomes, including delayed union (p-value=0.740), non-union (p-value = 0.888) or DSI. (p-value = 0.894) (Table 9-20).

Table 9-20. A summary of how the waiting time between a participant's injury and surgery influences the risk of the development of delayed union, non-union and deep surgical site infection

	Union n = 326	**Delayed union n = 69**	**p-value**
Time between surgery and injury (Hours: median: IQR)	68.0 (32.3-129.0)	75.6 (40-105.9)	0.740
	Union n = 372	**Non-union n = 23**	**p-value**
Time between surgery and injury (Hours: median: IQR)	69.7 (33.4-121.7)	59.5 (41.0-106.5)	0.888
	Deep surgical site infection n = 374	**Deep surgical site infection n = 21**	**p-value**
Time between surgery and injury (Hours: median: IQR)	69.3 (33.1-120.3)	65.0 (41.0-152.3)	0.894

CI – Confidence interval

IQR – Interquartile range

All CI are adjusted for clustering for multiple fractures per participant

9.2.6.10 Time of day of surgery: How this impacted the study outcomes.

Over half of the surgical procedures (229/395, 51.8%) took place outside of normal working hours (1700-0700) across both study sites. The proportion of participants who developed delayed bone union following a procedure performed out of hours was the same as those whose fracture went onto union (34/69, 49.3% vs 169/326, 51.8%). Similarly, in participants who developed DSI (12/69, 57.1% vs 191/374, 51.1%). However, slightly more non-union developed after surgeries were undertaken out of normal operating hours (16/23, 69.6% vs 187/372, 50.2%). Overall, for all time points, the time of day a fracture was operated on was not statistically significant for the development of delayed union, non-union or DSI in this study population (Table 9-21).

Table 9-21. A summary of how the timing of a participant's surgery influences the risk of the development of delayed union, non-union and deep surgical site infection.

	Union n = 326, %	**Delayed union n = 69, %**	**p-value**
Time of surgery			
Morning	76 (23.3)	22 (31.9)	0.300
Afternoon	81 (24.8)	13 (18.8)	
Out of hours	169 (51.8)	34 (49.3)	
	Union n = 372, %	**Non union n = 23, %**	**p-value**
Time of surgery			
Morning	94 (25.3)	4 (17.4)	0.200
Afternoon	91 (24.4)	3 (13.0)	
Out of hours	187 (50.2)	16 (69.6)	
	No deep surgical site infection n = 374, %	**Deep surgical site infection n = 21, %**	**p-value**
Time of surgery			
Morning	92 (24.6)	6 (28.6)	0.600
Afternoon	91 (24.3)	3 (14.3)	
Out of hours	191 (51.1)	12 (57.1)	

CI – Confidence interval

IQR – Interquartile range

9.2.6.11 Fracture site

Of the 395 IM nailings that were undertaken in the study population, 197 (49.9%) were in the tibia and 198 (50.1%) were in the femur. Participants who sustained a fracture of the tibia were more than six times more likely to develop a DSI (univariate OR 6.54 [2.17-28.20] p-value=0.001) compared to the femur. However, having a femur or tibia fracture was shown not to significantly affect the odds of participant developing a non-union (OR 1.10 [CI 0.47-2.60] p-value = 0.820) SSI (OR 2.03 [CI 0.39-14.8] p-value = 0.416) or late implant infection (7/7 infections all in the tibia). A summary of these findings can be seen in Table 9-22.

Table 9-22. A summary of how the fracture site influences the risk of the development of delayed union, non-union, deep surgical site infection, superficial surgical site infection and late implant infection

	Union n = 372, %	**Non union n = 23, %**	**Univariate odds ratio (95% CI)**	**p-value**
Fracture site				
Femur	187 (50.3)	11 (47.8)		
Tibia	185 (49.7)	12 (52.2)	1.10 (0.47-2.60)	0.820
	No deep surgical site infection n = 374, %	**Deep surgical site infection n = 21, %**	**Univariate odds ratio (95% CI)**	**p-value**
Fracture site				
Femur	195 (52.1)	3 (14.3)		
Tibia	179 (47.9)	18 (85.7)	6.54 (2.17-28.20)	0.001
	No superficial surgical site infection n = 389, %	**Superficial surgical site infection n = 6, %**	**Univariate odds ratio (95% CI)**	**p-value**
Fracture site				
Femur	196 (50.4)	2 (33.3)		
Tibia	193 (49.6)	4 (66.7)	2.03 (0.39-14.8)	0.416
	No late implant infection n = 388, %	**Late implant infection n = 7, %**	**Univariate odds ratio (95% CI)**	**p-value**
Fracture site				
Femur	198 (51.0)	0 (0)		
Tibia	190 (49.0)	7 (100)	NA[b]	NA[b]

CI – Confidence interval

IQR – Interquartile range

All CI are adjusted for clustering for multiple fractures per participant

b = not performed due to low number of values

9.3 Discussion – HOST 1 study

9.3.1 Principal findings

To our knowledge this is the first large, appropriately powered, prospective study to investigate fracturing healing in HIV-positive individuals. HIV was shown not significantly associated with the development of delayed bone healing following an IM nailing of the tibia or femur in this study population (univariate OR 0.76, [CI 0.37-1.44], p-value=0.417, multivariable OR 1.06 [CI 0.50-2.22], p-value=0.869). Anti-retroviral therapy regimen, CD4 count and viral load measurement at baseline and six months follow-up, were shown not to be associated with a risk of developing delayed union in the HIV-positive study population.

HIV-positive participants developed a statistically significant lower proportion of non-unions, compared to HIV-negative (univariate OR 0.16 [CI 0.01-0.78], p-value = 0.076, multivariable OR 0.17 [CI 0.01-0.92], p-value = 0.100), suggesting that HIV-positive status reduced the risk of developing a non-union following a fracture.

HIV-positive status was not associated with the development of post-operative DSI (univariate OR 1.96 [CI 0.72-4.89] p-value = 0.161, multivariable OR 2.59 [CI 0.86-7.80] p-value=0.090). There was a low number of participants who developed an SSI in the study population, making it difficult to draw any valid conclusions. However, the proportion of SSIs in HIV-positive participants was slightly lower (1.2% [1/83]), compared to 1.6% (5/312) in HIV-negative participants. Only 1.7% (7/395) of the study population developed a late implant infection. A higher proportion of HIV-positive participants developed late implant infection than HIV-negative (6.0%, 5/83 vs 06 %, 2/310).

9.3.2 HIV status in study population

The prevalence of HIV in the study population was 19.8% (71/358 participants). 41 out of 71 HIV-positive participants (57.7%) were taking ART on enrolment to the study and almost all of these participants were taking the same first line therapy - TDF, 3TC + ERV (38/41, 92.7%).

South Africa, with 0.7% of the world's population, accounts for 17% of the global burden of HIV infection.(52) The national prevalence of HIV is approximately 18.9%, but the prevalence in the Western Cape is much lower at 5.6%.(57), (58) Therefore, the prevalence of HIV in the study population was significantly higher at 19.8% (14.2% higher) compared to the Western Cape. This is potentially due to the fact that the majority of the study population were young adult males (273/358, 76.2%) living in and around the township areas of Cape Town. HIV is more prevalent in deprived areas of South Africa (420), such as townships. Young adult males are more likely to engage in high-risk behavior, potentially increasing the likelihood of HIV infection (421), (422) and be involved with crime (423),(424). As a result of their high-risk behaviour and high crime involvement, this study population represents a cohort of individuals that are at a high risk of suffering from trauma. In similar research from Malawi, researchers demonstrated a similar theme of a higher proportion of HIV in their study population of trauma participants when compared to the national prevalence, with the majority their study population being young adult males.(388), (389)

Approximately, 4.4 million people are currently receiving ART treatment in South Africa. This equates to 61% of the people living with HIV in the country.(53) This study cohort had a slightly lower proportion of participants taking ART on enrolment (41/71, 57.7%) compared to the national average. This increase at six-month follow-up to 69% (49/71) to be slightly higher than the national average. Current international targets are for 90% of those with HIV to be on ART.(425), (426) The aim was to have all participants on ART by 6 months follow-up. However, it is well

documented that young adult males have a low uptake of HIV services in South Africa and this was not possible in this study population.(422)

The proportion of delayed union in ART naïve participants was 23.3% (7/30) compared to 12.2% (5/41) in those participants taking ART on enrolment. Only one HIV-positive participant developed a non-union and they were on ART, making it difficult to draw any valid conclusions from the non-union data. However, since the proportion of delayed union was lower in those on ART, this might suggest that ART has little influence on the development of delayed union. This was further confirmed by the multivariable analysis which showed that ART was not a statistically significant factor for the development of delayed union in the HIV-positive study population. A much larger study would be needed to be appropriately powered to help understand the role of ART on fracture healing.

The overall median CD4 count and viral load were similar at six-months (413 cells/mm^3, IQR 302-653/2.13 log10 cps/ml (1.3-4.62), compared to baseline (402 cells/mm^3, IQR 265-630/1.99 log10 cps/ml, 1.30-410). In this study cohort, the median CD4 count on enrolment was 402 cells/mm^3 (IQR 265-630), which is the similar to published population based CD4 counts in South Africa.(427) Malaza et al showed the median CD4 count level in the HIV individuals on ART was 367 cells/µl (IQR 255–511 cells/µl) compared to 377 cells/µl (IQR 252–542 cells/µl) in ART naive group (p = 0.600) in a study in South Africa. (427)

Historically, researchers have recommended ensuring that the CD4 count is above 500 cells/µL prior to undertaking orthopaedic surgery, even emergency fracture fixation, due to high infection rates.(213) However, more recently researchers have suggested that the level of CD4 count makes little difference to post-operative complication rate following fracture surgery.(388) In those HIV-positive participants who developed DSI, there was no statistically significant difference in the CD4 count at baseline (median CD4 328 cell/mm3 [IQR 200-822]) compared to participants who

did not develop an infection (median CD4 417 Cell/mm3 [IQR 306-653]) on univariate analysis (OR 1.00 [CI 0.998-1.00] p-value = 0.394). Our study shows that a CD4 count less than 500 cells/μL appears to make no difference to the odds of developing post-operative DSI infection following internal fixation.

9.3.3 Comparison with the published literature

9.3.3.1 Whole study population

The overall delayed union rate in the overall study population was 17.4% (69/395), with a higher delayed union rate in the tibia fractures (23.4%, 46/197) compared to the femur (11.61 %, 23/198). Prior to commencing the study, it was anticipated that the delayed union rate in the overall study population would be 15%. This was used to formulate the sample size calculation for the HOST 1 Study, and this was based on previous research, the majority of which was from research in a high-income setting. (114), (124), (126), (406), (407), (428), (429)

There is little evidence currently available in the literature that accurately reports the risk of delayed union of tibia and femur fractures following internal fixation. This is likely due to the varying definitions of delayed union available in the literature and commonly researchers report delayed union rates that would be classified as non-unions by definition in this study.(131) Furthermore, the majority of research to date has focused on fracture non-union, rather than delayed union of fractures. This may be due to a number of factors. Firstly, it can be difficult to measure delayed union without the appropriate resources and protocols. It is labour intensive, having to capture data at set times before a fracture heals, since the majority of delayed unions do go on to heal, rather than form established non-unions. This is shown in this study cohort, where 66.6% (46/69) of delayed unions did not progress to non-unions. Additionally, the impact of non-union on an individual's and overall health and social

economic status is greater than that of a delayed union, making it a higher research priority.(430), (431)

Despite no clear and established definitions for delayed union for the tibia or femur in the literature, there are well-documented reports of differences in the union times of these two bones. (109), (114), (201), (432) The tibia takes longer than the femur to achieve bone union following a fracture, despite non-union rates being similar. (109), (114), (201), (432) A range of union times for the femur and tibia have been published. Approximate fracture union times of 12 weeks for the femur and 16 weeks for the tibia following a closed injury are commonly used.(114), (115) The higher proportion of delayed unions in the tibia may reflect the biological nature of the tibia and its normal healing process being longer to that of the femur. Therefore, due to the established differences in union times for both the tibia and femur, a more appropriate definition of delayed union for the femur should be shorter than that used for the tibia. This is acknowledged as a limitation of the study.

The non-union rate in the tibia was very similar to the femur (12/197, 6.0% vs 11/198, 5.5%) in the study population, with an overall a non-union rate of 5.8% (23/395) in the study population. This matches published data available in the literature which has shown non-union rates from 1.1-12.5% for femur and tibia fractures. (114), (116), (188), (431), (433)

The rate of deep surgical site infection (DSI) and superficial site infection (SSI) in the overall study population was 5.3% (21/395) and 1.5% (6/395) respectively. Recent literature indicates that the rate of post-operative SSIs in tibia and femur fractures, following fracture fixation, range from 3.4–4.2% and DSI rate of between 4.1-6.4%.(434) although, huge variations have been reported in the literature, from 2% to 88% depending on the area of the bone fractured and the fixation method used.(433), (435), (436), (437) Therefore, the rate of DSIs and SSIs in this study, mirrors that published in the literature in cohorts undergoing similar procedures.

DSI has been reported to be more common in the tibia (6.4% [95% CI, 2.4%-9.7%]) compared to the femur (5.7% [95% CI, 5.4%-8.8%]). (434) The reasons for this are multi-factorial, including different soft tissue coverage of the bone, blood supply and the types of injuries sustained in the tibia and femur.(438) Similarly to the literature, in this study population the proportion of DSI following a tibia fracture was 10.1 % (18/179) compared to 1.5% (3/195) in the femur. The higher DSI in the tibia fractures compared those reported in the literature could also be due to the slightly higher number of open tibia fractures compared to femur (36.4% [72/198] vs 32.9% [65/197]).

9.3.3.2 HIV-positive population

To our knowledge, no published research to date has accurately reported the outcomes of delayed union in HIV-positive individuals following a fracture. Harrison et al (20) and Babruam et al (374) reported delayed union outcomes in HIV-positive participants but in study cohorts of less than 7 participants. Therefore, due to these low numbers, delayed union comparisons cannot be drawn from the literature using other HIV-positive cohorts.

The proportion of non-unions in the study population was 5.8% (23/395), with a statistically significantly lower proportion of non-unions in the HIV-positive cohort (1.2% [1/83] vs 7.1% [22/312]), compared to the HIV-negative participants (univariate OR 0.161 [CI 0.01-0.78], p-value=0.076). Overall non-union rates of between 0 - 11% are reported in HIV-positive individuals following surgical fixation of a fracture. (213), (367), (382), (389), (394), (439),

The rate of non-union following an open fracture in the study population was 0% (0/23) compared to 10-43% (371), (370), (386) (440), reported in the literature. Therefore, the non-union rates in this study are similar to those already reported in closed fractures, but lower than those reported as open in HIV-positive participants.

The majority of the literature groups DSI and SSI into 'early implant infection' (infection within three months of surgery) and refers to 'late implant infection' as any infection six months or more from surgery.(213) The rate of 'early implant infection' in HIV-positive individuals following fracture fixation has been reported to range from 0-29 % in closed injuries(366), (367), (368), (369), (382) and 0-100% following an open fracture. (20), (213), (364), (370), (375), (393), When combining the number of DSIs and SSIs in the HIV-positive participants in this study to give a rate of 'early implant infection', HIV-positive participants had an infection rate of 9.6% (8/83). Therefore, the rates of DSI and SSI in HIV-positive participants in this study population were on the lower end of those reported in HIV-positive individuals in the literature.

The proportion of DSIs in the HIV-positive cohort was higher compared to the HIV-negative participants (8.4% [7/83] vs 4.5% [14/312]), (univariate OR 1.96 [CI 0.72-4.89], p-value=0.161) although the SSI rates were very similar (1.2% [1/83] vs 1.6 [5/312]) in the study population. As discussed in the previous section, literature indicates that in HIV-negative cohorts, the rate of postoperative SSIs and DSIs in tibia and femur fractures, following fracture fixation, ranges from 3.4–4.2% and 4.1-6.4% respectively.(434) Therefore, the rate of infection in HIV-positive participants in this study population is higher than the reported rate in HIV-negative individuals in the literature, as well as the study cohort.

A higher proportion of HIV-positive participants presented with late infections (6.0% [5/83] vs 0.6% [2/312]) compared to HIV-negative participants. The largest study to date reporting late implant infection, reported the outcome of late implant infection in 82 participants who had varying forms of fracture fixation. They reported late implant infection rates with a mean follow up of 25 months (12-52 months) of 0%.(388) Other smaller cohort studies suggested late implant infection rates of up to 18% but in smaller study cohorts. (387), (388), (439) However, in all studies to date, no appropriate control sample has been used. This study included a control sample and the late implant infection rates were reported to be higher in the HIV-positive

participants. Therefore, HIV appears to be associated with an increase in the odds of developing a late implant infection in this study population, but more research is needed.

9.3.4 Confounding factors

Age

The median age of the study population was 33 years (18-71 years). The odds of delayed union increased by 3% for every year increase in age at the time of fracture (univariate OR 1.02 [1.0-1.05] p-value 0.050, multivariable OR 1.03 [CI 1.00-1.06] p-value = 0.003) in the overall study population. Age was shown not to be a statistically significant factor for all other study outcomes of non-union, DSI, SSI or late infection. In vivo studies have suggested that fracture healing may be delayed in elderly animals,(141), (142), (143) but this has not been confirmed in clinical research and studies are conflicting.(144), (145), (116) This study suggests that age may influence the speed that a fracture heals but not the development of a non-union.

Sex

The majority of the study participants were male, 313 (78.3%) vs female: 87 (21.7%). This mirrors the expected cohort of individuals who are like to sustain trauma in the Western Cape. (73), (404), (441) Female participants were significantly less likely to have delayed union compared to male participants on univariate analysis (OR 0.38, [CI 0.16-0.78], p-value=0.014). On multivariable analysis, although delayed union was still less likely in the female population, this was borderline statistically significant (OR 0.41 [CI 0.17-1.01], p-value=0.053). Sex was shown not to be a significant factor for the development of non-union or DSI. Due to the high rate of male participants and the low number of infections, all SSIs were in males and so were the majority of late infections (5/7), making any conclusions difficult to draw from this data.

Currently, literature suggests that females are more likely to develop non-union compared to males. However, the evidence from these studies is in females over 55 years of age and following the menopause. These individuals have a lower level of circulating oestrogen, which plays an important role in promoting bone formation, stimulating anabolic and reducing catabolic processes.(59) In this study population, the majority of females in this study were younger than 55 years (91.8%, 78/85).

Worldwide, young adult males are more likely to suffer high energy injury.(441) Therefore, this could increase their risk of problems with fracture healing, since high energy injuries are more likely to develop fracture healing problems.(116) In this study population, 90.1% (246/273) of the male participants had an injury as the result of a mechanism that could be classified as high energy (motor vehicle accident, GSW fracture, pedestrian hit by vehicle, high energy fall), whereas 80.0% (68/85) of females had a mechanism of injury with a similar mechanism. This could therefore have contributed to the difference seen between men and women, although differences were not statistically significant.

Timing of surgery

The median time between a participant's injury and their surgery was 69.2 hours (33.6-121.7 hours). The time between injury and the date of surgery had no impact on any of the primary or secondary outcomes, including delayed union (p-value=0.740), non-union (p-value = 0.888) or DSI on univariate analysis. (p-value = 0.894). Just under three days (69.2 hours) is a relatively short period of time between injury and surgery. Long periods of time between injury and surgery have been associated with an increasing the risk of problems with fracture healing and infection. (178), (204), (442)

The majority of the surgical procedures (229/395, 51.8%) took place outside of normal working hours (1700-0700) across both study sites. The proportion of participants who developed delayed bone union following a procedure performed

out of hours was the same as those that had fractures that went onto union (34/69, 49.3% vs 169/326, 51.8%). The same was true for participants who developed DSIs (12/69, 57.1% vs 191/374, 51.1 %). However, the proportion of non-unions after surgeries undertaken out of normal operating hours (16/23, 69.6% vs 187/372, 50.2%) was higher than those undertaken in working hours, but this difference was not statistically significant (p-value=0.200).

With more than half of all procedures taking place out of normal working day hours, this surgical practice is unique to this setting. It is due to a combination of the high burden of trauma and resource restrictions at the study sites and in the South African health care system as a whole. If an operating theatre is available, then the orthopaedic team will use it to undertake a procedure. As a result, orthopaedic trauma procedures are performed 24 hours a day.

The vast majority of orthopaedic trauma practice in high income settings encourage minimal, if any, routine procedures to be undertaken out of hours. Health care systems actively encourage anything other than emergency procedures to be undertaken at this time of the day.(443), (444), (445), (446) There is set guidance on the management of pathology such as open tibia fractures out of hours in the United Kingdom.(447)

Overall, for all time points, the time of day a fracture was operated on was not a statistically significant factor for the development of delayed union, non-union or DSI in our study population. Although, there is a suggestion that a higher proportion of non-unions occurred in procedures performed out of hours. This may be due to the low number of participants that developed non-union and a study including a large sample size would answer this.

Vitamin D

The post-operative vitamin D level of the HIV-positive participants was slightly lower (41.25 vs 45.1 nmol/L) than HIV-negative, although this difference was not statistically significant (p-value = 0.85). On multivariable logistic regression analysis, the odds of non-union increased by 3% for every 1 nmol/L increase in vitamin D at baseline enrolment (multivariable OR 1.03 [CI 1.01-1.05] p-value = 0.009) in the overall population. However, high vitamin D level was not shown to be a risk factor for the development of delayed union on univariate (OR 1.01 (CI 1.00-1.02), p-value = 0.111) and multivariable analysis (OR 1.01 (CI 1.00-1.02) p-value = 0.181).

As discussed in chapter 4, HIV-positive individuals are at risk of vitamin D deficiency and ART also contribute to this. (274), (275), (276) Therefore, the lower level of vitamin D seen in the HIV positive participants compared to HIV-negative is to be expected. The National Institute for Health and Care Excellence define vitamin D deficiency as serum vitamin D levels less than 25 nmol/L.(448) Therefore, using this definition the median post-operative vitamin D level in both HIV-positive and negative groups was not within this range suggesting deficiency.

The role of vitamin D in fracture healing has, to date, been poorly investigated. There are a limited number of conflicting experimental studies reporting either a negative (146), (154) or no effect (155) of vitamin D deficiency on fracture callus formation and mechanical callus quality. (158) In this study, it is interesting that that higher levels of vitamin D was one of the only parameters to statistically result in impaired fracture healing. Currently, there does not appear to be any other clinical data to support these findings, although in vivo research has demonstrated similar results in rat and chick models.(449), (450) Therefore, this is an area of future research that requires further exploration.

Bone mineral density

A reduction in BMD is a common complication of HIV and its treatment and this has been established by a number of cross-sectional studies. (8), (9), (10), (11), (253), (254), (255), (256), (257) Additionally, ART, independently, has also been shown to decrease BMD and this will be discussed in more depth later on in this chapter.(253)

A total of 181 participants (45.25%, n=400) had their bone mineral density BMD measured using a Calscan DXL Densitometer in the study population. Twenty-seven out of 181 participants who underwent a measurement of their BMI were HIV-positive (15%). Confirming established research, there was a doubling of the rate of osteoporosis (T score > 2.5) in the HIV-positive cohort, compared to HIV-negative participants (2/27 [7.4%] vs 5/154 [3.2%]), but no statistical significance was found between the groups (p-value=0.5). The low numbers of participants meant that any valid conclusions are difficult draw from this data.

Socioeconomic status

A large proportion of the study population were living in the Cape Flats areas (250/342, 73.01%), an area of high unemployment and low socioeconomic status, compared to other areas of Cape Town Municipality.(451), (452) In the study population the unemployment rate was much higher than the regional average, at 52.5% (210/400), compared to 29.1% across the Western Cape.(412) Therefore, overall the study population appeared to be from a cohort of individuals with a high unemployment rate, living in a socioeconomically deprived area.

A higher crowding index, derived from the total household size divided by the number of living rooms, has been used to correlate with lower socioeconomic status.(415) The HIV-positive cohort had a slightly higher crowding index (1.5 [IQR: 0.25-4] vs 1.33 [IQR:0.25-7]), compared to the HIV-negative participants (p-value=(0.72). A higher proportion of the HIV-positive participants first sought medical treatment at a district level hospital (38/75, 50.7% vs 60/325, 18.5%).

District level hospitals generally serve the Cape Flats regions. Furthermore, the level of education was lower in HIV participants and the level of unemployment was also higher (46/75, 61.3% vs 164/325, 50.5%).

HIV-positive participants had a significant difference in albumin compared to HIV-negative individuals (36 vs 44 g/L, p-value = 0.02). Albumin has been used as an indicator of nutritional status. However it has been shown to poorly correlate with nutritional parameters and cannot be used independently to suggest any difference in the nutritional status between the HIV-negative and positive participants .(416)

Overall, although no formal poverty score was used in the study, the socioeconomic status of the HIV-positive cohort could potentially be interpreted as being lower than the HIV-negative group but none of these factors were shown to be risk factors for the development of any of the primary or secondary outcomes of the study.

The relationship between social deprivation and non-union is controversial. Although there is evidence to confirm that a lower level of socioeconomic status increases a individuals risk of fracture, (453) there is no reliable evidence to confirm socioeconomic status as clear risk factor for the development of delayed or non-union.(454) There is, however, a clear link between social deprivation and HIV infection.(455) Therefore, although the level of socioeconomic status may be lower in the HIV-positive participants, it is unlikely that this resulted in a significant factor that influenced fracture healing outcomes.

Mechanism of injury

The main mechanism of injury in the whole study population was due to road-related injuries (276/400 66.8%) and 21.5% (95/442 IM nailings) of injuries were due to gunshot wounds (GSWs).

There was a higher proportion of GSWs in the HIV-negative participants (23.9% [85/442] vs 11.15% [10/442]). However, there was a much higher proportion (49.4% [43/442] vs 32.7% [116/442]) of HIV-positive participants injured after being hit by a vehicle as a pedestrian, compared to HIV-negative participants. Additionally, more of the injuries in the HIV participants were due to road-related injuries (70.1% [61/442] vs 60.6% [215/442]).

If the mechanisms of injuries (high energy injuries, MVA (car and pedestrian), GSW fracture) that are more likely to result in a high energy fracture are grouped together, there was a similar proportion of these mechanisms of injury in the HIV-positive and negative groups (74/87 85.1% vs 317/355 89.4%).

Interestingly, six participants who were enrolled following a GSW fracture, were shot dead within six weeks of enrolment in the study. Therefore, there was a 6.3% (6/95) chance of a participant, who was enrolled in the study following a GSW being dead, within six weeks of their injury. This highlights the high crime and dangerous environment a number of the study participants are living in.

Open fracture

- **Study population**

There were 137/395 (34.7%) IM nailings in 358 study participants with open fractures. The proportion of delayed union and non-union rate in the open fracture study population was 28.5% (39/137) and 10.9% (15/137). The DSI rate was 8.8% (12/137); 3.6% (5/137) of open fractures developed an SSI and 2.1% (3/137) developed late implant infection.

It is well established that open fractures result in higher rates of non-union and post-operative infections when compared to closed injuries and the outcomes are determined by the severity of the open fracture and Gustilo Anderson grade.(210), (456), (457), Multiple studies reported infection rates following intramedullary

nailing of open tibia fractures. Court Brown reported deep infection rates ranging from 1.8% to 12.5% and other research has demonstrated rates ranging from 1.9 to 18.2%. (117), (162), (200), (407), (458), (459) Similar, SSI rates have also been reported.(117) Following an open tibia fracture, non-unions have been reported to occur in between 2.9-21.5% of study populations.(114), (117), (126), (200), (210), (407) The rate of infection in open femoral shaft fractures undergoing intra-medullary nailing is slightly lower at 4.8–5.6 % and non-union rates of 4.8–14.1%.(196), (460), (461), (462) Again, SSIs and DSIs are commonly grouped together and no clear definition is given.

In the United Kingdom (UK) there are set standards and guidelines for the management of open tibia fractures, which are used for the management of all open fractures.(447) In South Africa, although the aim is to undertake similar management as those set in the United Kingdom, or similar, this is commonly not possible due to the high burden of trauma and resources available. An example is that the UK guidelines recommend debridement surgery within 12 hours for high energy injury or 24 hours for a low energy injury. This is simply not possible at the two study sites.

A high proportion of participants underwent IM nailing as a single procedure, without an initial washout, or application of external fixator (88.91%, 143/161), having their definitive surgery at a median 59.5 hours (27.15-106.25), 2.51 (1.13 – 4.42) days. This is nearly 1.5 days longer than the UK guidance.

GSW fractures are also managed very differently at the study sites, compared to the UK. These fractures in the UK would be managed like any other open fracture, with surgery aimed for within 12-24 hours. Whereas at both study sites, all low velocity GSW fractures have their bullet entry and exit wounds left to heal by secondary intention and the fractures are treated as a closed injury, with surgery not expedited. Despite this, number of participants in this study population that developed post-operative infection and non-union following an IM nailing for an open tibia or femur

fracture mirror the published literature. Few studies have reported the outcomes of delayed union therefore comparisons are not possible.

- **HIV-positive population**

Following an open fracture, HIV was not associated with the development of delayed union (OR 0.93 [CI 0.31-249] p-value = 0.89), non-union (0 non-unions in HIV-positive participants) or DSI (OR 1.86 [CI 0.39-6.91] p-value = 0.38) and SSI (OR 1.32 [CI 0.66-9.50] p-value = 0.807). Although, on univariate logistic regression analysis the association between HIV and late implant infection was borderline significant (OR 11.40 [1.05-25.20] p-value = 0.051), caution should be made when interpreting this result due to the low numbers.

There is little evidence surrounding the risk of delayed union and non-union following an open fracture in HIV-positive participants in the currently literature but non-union rates of between 10-43% have been reported in a small number of studies. (20), (389), (393), (440) Whereas, the rate of non-union this study was much lower (0%). However only 15 participants developed a non-union following an open fracture and definitive conclusions cannot be drawn from these low numbers.

It has been suggested that HIV is a risk factor for early and late implant sepsis in open fractures, with early infection rates reported to be between 0-100% and late infection rates of 0-8%. (20), (213), (370), (371), (386), When reporting proportional comparisons SSI, DSI and late implant infection according to HIV status in open fractures, due to the low number of participants who developed these outcomes overall, it is difficult to draw any valid conclusions from this data. However, the proportions of HIV-positive participants who developed post-operative infections following fracture fixation for an open fracture were higher in HIV positive participants (DSI - 13.6% 3/22 vs 7.8% 9/115), SSI - 4.5% 1/22 vs 3.5% 4/115) late implant infection - 9.1% 2/22 vs 0.9% 1/115). This highlights an important area of future research.

9.3.5 Limitations of this work

One of the main limitations of this study is that it included fractures at two injury sites, the tibia and femur. Fracture site was shown to be a statistically significant risk factor for the development of DSI and delayed union. Therefore, ideally the study should have been undertaken using only one injury site. Additionally, if a participant had more than one fracture, both fractures were entered as two sperate injuries.

Seventy-one HIV-positive participants who underwent 83 IM nailings were included in the final analysis. 69% (49/71) were taking ART at six-month follow-up. It has been suggested that ART negatively impacts the rate of fracture healing (Professor Hamish Simpson – personal communication of unpublished in vivo research). Ideally in order to determine the effect of HIV on fracture healing, the study should have only included those participants who were not on ART therapy. Or alternatively, only participants on ART could have been enrolled. However, due to ethical restrictions and time limitations of the study, this was not possible. Including a mix of participants on ART and ART naïve individuals makes it more difficult to draw any conclusions to from these limited in numbers. Alternatively, a large sample size of participants could have enabled this to be investigated.

Participants had the date they first received a positive laboratory blood sample for HIV and the date of their IM nailing surgery recorded. Therefore, it was possible to determine the length of time a participant had been diagnosed with HIV. However, it is recognised that this is only an estimate and that it is not possible to determine the exact date a participant was diagnosed with HIV. This is because HIV diagnosis can be made from a finger prick test. Individuals are then offered a formal blood sample test to confirm this diagnosis (Chapter 7). The results of finger prick testing are not recorded anywhere on South Africa's health care records online software. Only a blood sample tests recorded on the national blood sampling laboratory records were used in this study to confirm the date of HIV diagnosis. Therefore, a participant may

have been diagnosed with HIV for a longer period than that recorded in this study. Additionally, even if the length of time a participant has been diagnosed with HIV is accurate, this does not give an indication of the length of time they have been living with HIV, since it is rarely possible to determine when someone was actually infected with the virus.

The length of time a participant was taking ART was recorded and again this was an estimate, since on occasions, participants could not fully recall the exact date they started their medication. This information was also reliant on the participant telling the truth since it is possible that they were saying that they were on ART but no assessment of if they were actually taking it was made.

The GA grade was determined by the operating surgeon at the time of first debridement of the open fracture. In 88.91% (143/161) of all open fractures, this was done by the operating surgeon who undertook the definitive IM nailing and the first debridement at the same time. In 11.08% (18/161) of cases the primary debridement was done prior to definitive IM nailing. Therefore, the GA grade was determined following a discussion with the surgeon who undertook the primary debridement and documented by the surgeon who undertook the definitive IM nailing on the surgical study proforma. Ideally two surgeons, blinded to HIV status, should have made the assessment of the participants' GA grade. The studies' ethical approval meant that participants could not be enrolled until IM nailing had been undertaken and due to resource limitations, having two blinded reviewers was not possible. It is important to highlight that 51.8 % (229/395) of procedures were performed out of working hours, resulting in challenges to this potential method. Despite this, it is recognised that this could have introduced errors when reporting the GA grade.

No formal socioeconomic scoring system was used to determine socioeconomic status of the participants. Therefore, it was not possible to accurately determine the participants social status. Those in a lower socioeconomic class are potentially at risk

of problems with fracture healing and infection, although there is currently no established evidence to confirm this. However, due to the fact this was not measured, potentially it could result in a confounding factor that was not taken into account during the analysis.

The lost to follow-up rate in the study was 10.5% (42/400), which is very good in the study setting, since the follow-up rate of patients at GSH following an operative orthopaedic surgery is less than 40% in routine clinical practice.(73), (404) It is recognised that the systematic differences between those with and without loss to follow-up could have had an impact on the study outcomes but it is unlikely this low rate could have an impact on the results.

The main study outcome was delayed bone union. In order to determine the true effect of HIV in fracture healing, non-union should have been used as the study's primary outcome. One of the reasons it was not was that non-union was is such a rare outcome. In order to complete a similar study powered for the outcome of non-union, a significantly higher number of participants would need to be enrolled. Due to logistical, time and financial resources this simply was not possible. In order to confirm this, an appropriately powered study would need to be undertaken. The same is true for the other outcome measures of DSI, SSI and late implant infection.

The RUST score was used to determine the primary outcome of delayed union. This scoring system has not been validated for use in the femur. However, it is the best tool available to determine bone union without the need for additional investigations, such as computer tomography (CT) scanning.(129) GSH and TSH have one functioning CT scanner and a waiting list of over six months to use it. Therefore, logistically using this would not be possible. However, the RUST score relies on human interpretation, therefore, it could potentially to introduce errors and/or bias.

9.3.6 Summary

HIV was not shown to be associated with the risk of developing delayed bone healing following an IM nailing of the tibia or femur in our study population. However, the proportion of HIV-positive participants who developed non-union was statistically significantly lower than in HIV-negative equivalents. Finally, HIV-positive status was not shown to be a statistically significant risk factor for the development of deep surgical site infection in this study population.

CHAPTER 10. HIV IN ORTHOPAEDIC SKELETAL TRAUMA 2 STUDY: Aims, Design, Methodology and a Descriptive Analysis of the Study Cohort

10.1 Aims of this chapter

In this chapter, a description of the background, aims, methods and analysis of the of the HIV in Orthopaedic Skeletal Trauma (HOST) 2 study cohort will be presented. The second of two linked studies that investigate the effect of HIV infection on fracture healing.

10.2 Introduction

There have been no previous case-control studies that have been undertaken to date investigating the effect of HIV on fracture healing. As discussed earlier in Chapter 5, it is difficult to draw any valid conclusions from the studies available in the literature to date regarding the true effect of HIV infection on fracture healing. These findings prompted this study.

The hypothesis underlying this research is that HIV infection is a risk factor for the development of non-union following a fracture of the femur or tibia.

10.3 Aims and objectives

The aim of HOST 2 was to establish whether HIV infection is a risk factor for the development of non-union following a fracture of the femur or tibia.

The main objectives of the HOST 2 Study were:

1. To establish whether HIV infection is a risk factor for the development of non-union following a fracture.
2. To investigate other risk factors associated with non-union in HIV-positive and HIV-negative adults.

10.4 Methodology

10.4.1 Study design

This was a multi-centre, case-control study of participants presenting with non-union of fractures over a 16-month period within the Orthopaedic and Trauma Department at GSH and TBH, Cape Town, South Africa. Participants presenting with non-union following a fracture of the tibia or femur shaft were matched to a control group of participants with healed fractures.

10.4.2 Study site

Participants were enrolled from the orthopaedic department of two tertiary referral trauma hospitals, GSH and TBH, in Cape Town, South Africa.

10.4.3 Study population

Cases – All participants older than 18 years of age with established non-unions of the femur or tibia shaft, following any form of operative or non-operative management, were potentially eligible for inclusion in the study.

Controls – All participants older than 18 years of age with closed or open tibia and femur fractures who underwent operative or non-operative management that went on to heal within six months of injury were potentially eligible for inclusion in the study.

10.4.4 Definitions and study outcomes

The definitions used in the HOST 1 study were also applied to this study. Definitions to describe “union” and “non-union” of a fracture were as follows:

Union:

• Radiological union on RUST score (score of three on at least three cortices in AP, lateral, medial or posterior cortex – a total of nine or more) within six months of surgery.(125), (126), (127), (128)

Non-union:

One or both of the following:

- Impaired bone healing at nine months on RUST score (RUST score < 9). (125), (126), (127), (128)

Fracture non-unions were categorised into two main types:

- **Hypertrophic**: The primary factor influencing non-union of the fracture is likely to be a mechanical problem with the form of treatment.(210)
- **Atrophic/oligotrophic**: The primary factor influencing non-union of the fracture is likely to be a local and/or systemic biology.(210)

The following definitions were used to determine the type of non-union on x-ray:

Hypertrophic non-union: one or both of the non-union classification parameters above plus callus formation around fracture with an elephant’s foot or horse’s hoof appearance.(463)

Atrophic/oligotrophic non-union: one or both of the non-union classification parameters above plus no callus formation around the fracture site or periosteal reaction while minimal callus formation.(463)

The following primary and secondary outcomes were used:

Primary study outcome:

- The odds ratio of femur or tibial fracture non-union, comparing HIV-positive to HIV-negative participants after adjustment for important confounding factors.

Secondary study outcomes:

- Risk factors associated with non-union in HIV-positive and -negative adults.
- Differences in the Disability Rating between participants with and without fracture non-union.

10.4.5 Inclusion and exclusion

The criteria used to determine if a participant was eligible for inclusion in the study were as follows:

*Inclusion criteria – **Cases***

Participants were eligible for HOST 2 Study if they:

- were older than 18 years of age
- sustained a previous closed or open fracture of the tibia or femur (metaphysis or diaphysis)
- had a single course of management for their fracture (e.g. conservative only, operative only)
- developed a non-union of the tibia or femur shaft

*Exclusion criteria – **Cases***

Participants were excluded from participation in this study in the case of:

- major head injury
- pre- or post-surgical infection at the fracture site

- severe burns
- pathological fracture
    - fractures that resulted from low energy injuries that occurred through an area of bone weakness with a pre-existing abnormality
- previous non-union of a fracture that had already healed prior to enrolment in the study
- intertrochanteric femur fractures
- intra-articular fractures
- evidence that the participant would be unable to adhere to study procedures and complete questionnaires

Inclusion criteria – ***Controls***

Participants were eligible for HOST 2 study if they:

- were older than 18 years of age
- sustained a closed or open fracture of the tibia or femur shaft
- fracture had healed within six months of injury

Exclusion criteria – ***Controls***

- major head injury
- pre- or post-surgical infection at the fracture site
- severe burns
- pathological fracture
- intertrochanteric femur fractures
- intra-articular fractures
- fracture that had not healed within six months
- evidence that the participant would be unable to adhere to study procedures, complete questionnaires

10.4.6 Matching, screening and enrolment

10.4.6.1 Matching

Cases were matched in a 1:1 ratio with controls on the following criteria:

a) Age: + / – 10 years
b) Sex:
    - Male
    - Female
c) Injury:
    - Tibia
    - Femur
d) Management of fracture:
    - IM nailing
    - Open reduction and internal fixation with plate and screw fixation
    - Ilizarov external fixator frame
    - Taylor-spatial external fixator frame
    - Conservative management

One ‘case’ was matched with a single ‘control’. Once a ‘control’ had been enrolled, they were not eligible for matching with another ‘case’.

10.4.6.2 Screening and enrolment

Cases

All 'case' participants were identified by the following four methods:

1. **Weekly limb reconstruction outpatient clinics at both base hospitals**

One senior fellowship trained surgeon (Maritz Laubscher (ML) at GSH, Nando Ferreira (NF) at TBH) was the sole provider of orthopaedic care for all lower limb non-union cases in that hospital at the time of the study. Any participant who developed a non-union of the tibia or femur were referred to these surgeons for an opinion on ongoing management. ML and NF screened all their limb reconstruction clinics on a weekly basis and if an eligible participant was identified, they informed the research nurses and the participant was reviewed the same day for consideration for recruitment. If ML or NF was absent from clinic due to sickness or holiday, there were clear instructions left with their surgical fellow to do the same in their absence. ML and NF are co-investigators on the HOST 1 and 2 Study.

2. **Weekly elective orthopaedic limb reconstruction surgery admissions to both study site hospitals**

The two surgeons (ML and NF) were also the only surgeons at the study site hospitals (TBH and GSH) who provided an operative service for participants with non-unions to the tibia or femur at the time of the study. Thus, any participant who was missed by procedures described above was subsequently enrolled if they were admitted for surgery at GSH and TBH. The research nurses screened the weekly planned operating lists for ML and NF. If a participant was identified from a pre-planned elective operating list, the participants were considered for enrolment by the research nurse team on the day of admission for surgery.

3. **Prospective daily fracture clinics**

All orthopaedic consultants and trainees, at both GSH and TBH, screened all fracture clinics on a daily basis for any participant who had a non-union to the femur or tibia.

If a potential participant was identified, the research nurses reviewed them and considered them for enrolment on the same day.

4. HOST 1 Study database

All HOST 1 Study enrolled participants were deemed eligible for recruitment into the HOST 2 Study. Once a HOST 1 Study participant reached their primary outcome and the fracture had developed a non-union, the participant was reviewed by the research team and considered for enrolment into the HOST 2 Study.

Participants were recruited prior to treatment of their fracture non-union. When a 'case' participant was deemed eligible for recruitment and a diagnosis of non-union was established, the research nurses were informed via mobile phone or a picture of the participant's name and current location was uploaded to an encrypted smartphone application. Following screening by the research nurses the participants were either enrolled or excluded from the study. If there were any concerns regarding eligibility for the study, I personally reviewed the participant with the research nurses.

All participants were recruited on the day they were identified and underwent an x-ray of their non-union site.

Controls

Once a participant was enrolled as a 'case', the study team commenced recruitment to identify an appropriate 'control' participant using the previously mentioned matching criteria above.

Control participants were identified by the following three methods:

1. **Daily fracture clinics**

All consultants and orthopaedic trainees, at both GSH and TBH, were given a weekly screening log sheet explaining the type of 'control' participant the research team were currently trying to identify (Figure 10-1).

Figure 10-1. Example of weekly screening log used to identify 'control' participants

HOST Study

Screening log – 4.3.19

Male	52	Femur	Conservative
Female	27	Femur	ORIF
Female	71	Tibia	ORIF
Male	34	Tibia	Ilizarov frame
Male	29	Tibia	Ilizarov frame
Male	39	Tibia	Ilizarov frame
Male	22	Tibia	Ilizarov frame
Female	35	Tibia	Ilizarov frame
Male	27	Tibia	Ilizarov frame

The surgeons then screened all daily fracture clinics for any participant who met the criteria for recruitment according to the screening log (Figure 10-1). If a potential participant was identified who met the four criteria of a 'control' participant, the research nurses were informed using an encrypted smartphone application and the participant was reviewed the same day for consideration for enrolment.

2. **Daily orthopaedic trauma meeting**

During the daily trauma meeting Sithombo Maqungo (SMG) or ML went through the weekly matching screening log with all the orthopaedic trainees responsible for the daily care and operations for all orthopaedic in-patients. Any participants identified were then considered for enrolment by the research team. During weekends or holidays, screening commenced on the following working day and any participants missed were identified.

3. **HOST 1 Study database**

All HOST 1 Study enrolled participants were deemed eligible for matching process. Using the weekly screening log, once a HOST 1 Study participant reached their primary outcome and the fracture had united, they were screened to see if they matched any of the cases recruited in the HOST 2 Study. If they were matched, the research team recruited them to the HOST 2 Study.

The sampling frame comprised of participants with confirmed fracture union. Participants were recruited at the following time points:

a) First presentation following a fracture
b) Partway through treatment for a fracture
c) Final presentation for a healed fracture

Participants who were recruited at first presentation of a fracture or partway through treatment were followed up until the fracture united. X-rays were performed on recruitment and when the participants fracture healed.

Case and controls

The case and control participants underwent a baseline questionnaire, and established risk factors for non-union were recorded (Table 10-1). Data collected included: demographic details; injury and surgery details; clinical history (including co-morbidities, previous history of tuberculosis); smoking and alcohol use; HIV status

and history; domestic situation (including household size, number of young children in household, number of rooms in dwelling); and socioeconomic status. Socioeconomic status was defined by level of education attained, employment, asset ownership, access to cooking facilities, water and electricity, type of sanitation facility and housing type.

The GA grade was determined retrospectively from the medical notes, or via communication with the surgeon who undertook the primary debridement following an open fracture. On all occasions a GA grade was able to be determined from one of these methods.

The participants' full blood count, renal function, vitamin D level and albumin were assessed. All participants underwent HIV testing; CD4 cell counts and HIV-1 viral load were evaluated if HIV-positive. If the participant was already known to be HIV-positive (available laboratory confirmation), their ART regimen was recorded, if appropriate.

Bone healing was assessed using a validated x-ray scoring system – the Radiological Union Scale for Tibial Fractures (RUST scoring system).(123), (124)

Table 10-1 Full visit assessment schedule

Classification	Study measurement
Predictor	HIV status/test
Predictor	HIV investigations (CD4 count, viral load if applicable)
Risk factor	ART – if applicable
Risk factor	NSAID and steroid use
Risk factor	Other lab investigations (FBC, renal function, albumin, Vitamin D)
Risk factor	Socio-demographic characteristics & medical history
Risk factor	Body Mass Index
Intervention	Injury & treatment detail
PROMS	Disability Rating Index
Main outcome	RUST score

ART – Anti-retroviral therapy

HIV – Human immunodeficiency virus

NSAID - Nonsteroidal anti-inflammatory drug

FBC - Full blood count

RUST - Radiographic Union Scale for the Tibial Fractures

10.4.7 Determining the primary outcome measure and x-ray parameters

Bone healing was assessed using a validated x-ray scoring system – the Radiological Union Scoring system for Tibial Fractures (RUST scoring system).(123), (124) Two independent observers (Maritz Laubscher (ML) and Pravesh Panchoo (PP): both orthopaedic surgeons), blinded to HIV status, assessed radiological fracture union. All participants' identifiable information was removed from the x-rays prior to viewing and no laboratory results were made available for them to assess HIV status. Both readers independently reviewed the x-rays of each participant once an outcome of union or non-union was confirmed. If there was a disagreement between the two reviewers regarding the outcome using the RUST score, a third reviewer (Professor Michael Held (MH), orthopaedic surgeon) independently reviewed the x-ray and

assessed fracture union using the RUST score. Whichever RUST score outcome the third reviewer made (union or non-union) resulted in the final outcome decision for the participant.

The method of scoring using the RUST score was the same as decided in Chapter 8 for the HOST 1 study.

Both ML and PP independently assessed the x-rays of cases to determine the type of non-union each participant had established: hypertrophic or oligotrophic/atrophic. If there was disagreement between the reviewers regarding the type of non-union, a third reviewer, MH, independently reviewed the x-ray and the outcome that was agreed by two of the three reviewers was the final outcome. No cases had more than one fracture with a non-union. On no occasion did the reviewers disagree on the non-union type.

10.4.8 Study procedures

10.4.8.1 Laboratory investigations

The investigations that were performed on enrolment are listed in Table 10-2.

Table 10-2 Enrolment laboratory investigations

Blood parameter
Haemoglobin (g/dL)
White blood cells (x 10^9 / L)
Creatinine (umol/L)
Urea (mmol/L)
Albumin (g/L)
Vitamin D (nmol/L)
HIV Test

10.4.9 Follow-up

Following initial enrolment, all cases did not require any follow-up. If a 'control' participant was recruited at first presentation of an injury or was partway through treatment of a fracture, the participant was followed-up until union was established before six months. The 'control' participants that were recruited prior to fracture union, all their fractures healed by six months or sooner. Once bone union had been confirmed, they were added to the HOST 2 Study database. 'Control' participants were reviewed according to the below follow-up protocol, up until the fracture healed, when they were discharged. The participants received reimbursement for their travel to attend clinic as follows in Table 10-3.

Table 10-3 Participant reimbursement schedule

Time point	**Reimbursement amount – exchange rate 19.6.2019**
2 weeks	150 Zar - £8.25
6 weeks	150 Zar - £8.25
3 months	150 Zar - £8.25
6 months	200 Zar - £11

10.4.10 Patient reported outcome measures (PROMS)

The following PROMS were collected at enrolment.

Disability Rating Index (DRI) – Appendix 13-1

The DRI is a self-administered, 12-item Visual Analogue Scale questionnaire assessing the participants' own rating of their disability.(403) This measure was chosen as it addresses 'gross body movements 'rather than specific joints or body segments. Therefore, it facilitates the assessment of participants with different fractures and injuries of the lower limbs. The closer the score is to zero, the better a participant's 'gross body movement'.

10.4.11 Completion of the study

Participants who still needed follow-up assessments as part of the clinical management continued to be treated by the Trauma and Orthopaedic Surgery Unit, until their condition had stabilised to a point where no further medical intervention was required, and they were discharged.

10.4.12 Withdrawals

Participants who were recruited but declined to continue to take part in the study at any time were withdrawn without prejudice. A decision to decline consent or withdraw did not affect the standard of care the participant received and they continued standard hospital follow-up care.

Participants had two options for withdrawal:
1) Participants withdrew from completing any further questionnaires but allowed the study team to still view and retain, anonymously, any relevant hospital data that was recorded as part of normal standard of care, e.g. x-rays and further surgery information.

2) Participants withdrew wholly from the study, but data obtained up until the point of withdrawal was included in the final analysis of the study; thereafter, no further data was collected for that censored participant.

10.4.13 Adverse events

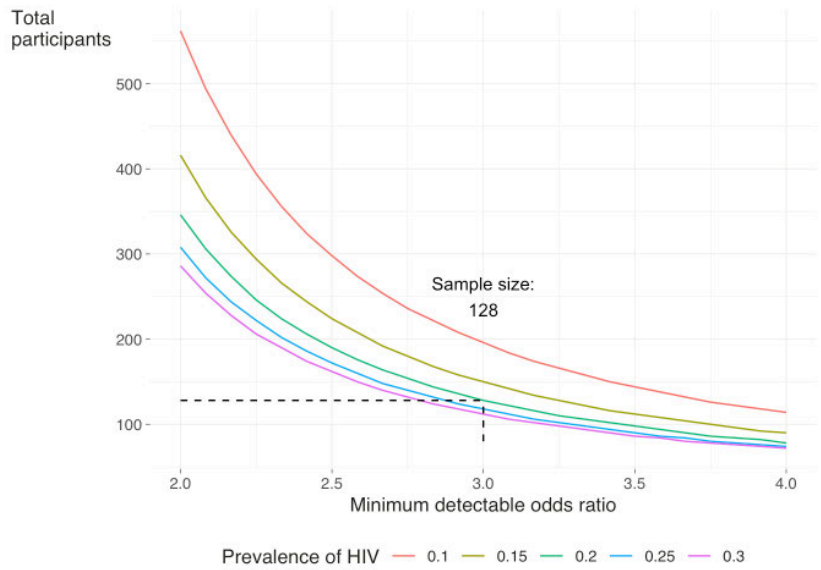
Serious adverse events (SAE) were defined as any serious untoward medical occurrence in a clinical study subject and which did not necessarily have a causal relationship with the treatment. These included any untoward and unexpected medical occurrence that: ‘results in death’, ‘is life-threatening’, ‘requires

hospitalisation or prolongation of existing in-patient ´hospitalisation', 'results in persistent or significant disability or incapacity', 'is a congenital anomaly or birth defect' or 'any other important medical condition which, although not included in the above, may require medical or surgical intervention to prevent one of the outcomes listed'. These were recorded in on the electronic 'Redcap' database.

10.4.14 Sample size calculation

Sample size calculation for the case control study used the methodology described by Dupont, (464) and included the Fleiss (465) correction for matched case-control design. Assuming that 20% of the controls would be HIV-positive (388), (368), a total sample size of 128 (64 cases and 64 controls) would give 80% power to detect at least an odds ratio of 3.0 for non-union, comparing between the case and controls groups (Figure 10-2).

Figure 10-2 Sample size calculation



10.4.15 Statistical analysis

Distributions of baseline characteristics were summarised using means, medians, proportions and distributional measures (standard deviations and interquartile ranges), and tabulated and plotted and compared between the exposure (case) group and non-exposure group (control).

1. For the primary outcome (non-union), confirmation was made that there were sufficient matching strata between the case and controls by cross-tabulation.(466) A multivariable logistic regression model was then constructed to estimate the odds ratio and 95% confidence interval for non-union comparing between case and control participants and adjusting for matching characteristics, and additional important confounders identified *apriori* through construction of putative causal diagrams. A separate model was constructed for HIV status of participants only to estimate the associations between HIV status on the outcome.
2. For the secondary outcomes, a similarly multivariable logistic regression model was constructed to estimate the odds ratio and 95% confidence interval for non-union comparing between HIV-positive and HIV-negative participants and adjusting for important confounders.

Missing data was reported in tables of baseline characteristics. In constructing multivariable models, we compared estimates obtained from complete case analysis and following multiple imputation of missing values.

In order to construct the HOST 2 study univariate and multivariable logistic regression models, the same method was used as described in Chapter 8 for the HOST 1 study.

Analysis of difference between continuous and categorical data not using logistic regression analysis, was assessed using t-test or chi-squared test respectively.

All statistical analysis was undertaken using ‘R’ statistical computing software.

10.4.16 Study timescale

The study schedule for the duration of recruitment and follow-up up was as follows: January 2018 – April 2019 (14 months) – subject recruitment and any follow-up took place at GSH and TBH

10.5 Results: Descriptive analysis of HOST 2 study cohort

10.5.1 Recruitment

From December 2017 until April 2019, 83 participants were identified as established non-unions of the tibia or femur and were screened and considered for inclusion in the study. cases were matched in a 1:1 ratio with controls on the following criteria:

a) **Age: + / – 10 years**
b) **Sex**:
    i. Male
    ii. Female
c) **Injury**:
    i. Tibia
    ii. Femur
d) **Management of fracture**:

20 participants did not meet the study inclusion criteria and were not enrolled. (Figure 10-3) The predominant reason for participants not being enrolled was that the they had infected non-unions (15/20, 75%) or participant refusal to participant in the study (5/20, 25%). Six HIV-negative, participants were excluded since it was not possible to identify matched controls. (Table 10-4) The full breakdown of the exclusion criteria and the number of participants excluded from enrolment can be found in Figure 10-3.

Figure 10-3 Flow diagram of study population recruitment

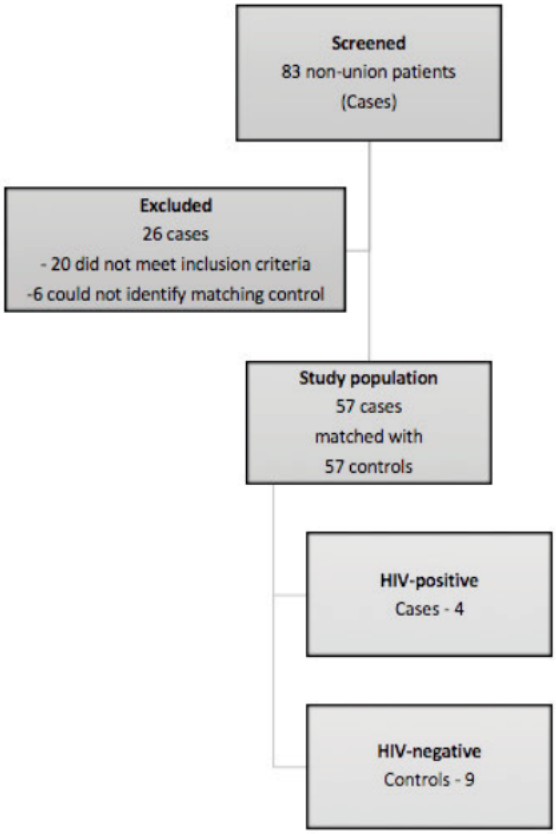


Table 10-4. Patients screened but not included in final cohort since a matching control could not be identified

Patient	Hospital	Sex	Age (years)	Fracture site	Fracture Class	Management	Reason for not matching
1	TBH	Male	52	Femur	33-A1	Conservative	Age
2	TBH	Female	71	Tibia	43-A1	ORIF	Age
3	GSH	Male	59	Tibia	43-A3	Conservative	Age
4	TBH	Female	60	Tibia	42-A2	Ilizarov frame	Sex
5	TBH	Female	28	Tibia	43-A3	Ilizarov frame	Sex
6	TBH	Female	40	Tibia	42-C3	Ilizarov frame	Sex

GSH – Groote Schuur Hospital

TBH – Tygerberg Hospital

10.5.2 Baseline characteristics

The main study cohort of 57 cases were matched with 57 ‘control’ participants. They were recruited between January 2018 and April 2019. Nineteen of the 57 ‘case’ participants and 36/57 of the ‘control’ participants were recruited from the HOST 1 Study.

The majority of the participants were male (44/57, 77.2 % vs 13/57. 22.8%), with a median age of 36 years in each group. 37 tibia and 20 femoral nails were enrolled and the main form of treatment for the fractures was IM nailing (36/57, 63.2%). There were 16 circular fixators (28.1%) enrolled, three fractures managed with open reductions and internal fixations with plates and screws (5.3%) and three (3.5%) fractures treated conservatively.

There was a similar proportion of smokers in the ‘control’ compared to the ‘case’ group (26/57, 45.6% vs 23/57, 42.1%), with no statistical significance between the groups (p-value=0.7).(Figure 10-4) As expected, the disability rated index (DRI) index was lower in the ‘control’ group, when compared to the cases (24.5 vs 45.2, p-value=0.001).(Figure 10-5) The basic demographics and characteristics of the study participants are summarised in Table 10-5.

Table 10-5. Baseline characteristics of study population

Characteristics	Cases (Non-union) N = 57 (%)	Controls (Union) n = 57 (%)	p-value
Sex			
Male	44 (77.2)	44 (77.2)	1.00
Female	13 (22.8)	13 (22.8)	
Age (yrs: median, IQR)	36 (21-76)	36 (18-63)	0.530
Fracture site			
Tibia	37 (63.2)	37 (63.2)	1.00
Femur	20 (36.8)	20 (36.8)	
Management			
Plaster	3 (5.3)	3 (5.3)	1.00
IM nailing	36 (63.2)	36 (63.2)	
Plate and screw fixation	2 (3.5)	2 (3.5)	
Circular fixator	16 (28.1)	16 (28.1)	
Hexapod(Taylor spatial)	4 (25.0)	4 (25.0)	
Ilizarov	12 (75.0)	12 (75.0)	
Alcohol			
Yes	24 (42.1)	25 (43.9)	0.99
No	33 (57.9)	32 (56.1)	
Smoking			
Non smoker	23 (40.4)	26 (45.6)	0.577
Smoker	34 (59.6)	31 (54.4)	
Cigarettes per day[a]			
0-5	8 (23.5)	7 (22.6)	0.600
6-10	13 (38.2)	14 (24.6)	
11-20	8 (23.5)	7 (22.6)	
>20	5 (14.7)	3 (5.3)	

Duration of smoking history			
(cases n=34, controls n = 31)			
< 1 year	0	0	0.912
1-5 years	3 (8.8)	4 (12.9)	
5-10 years	7 (20.6)	11 (35.5)	
> 10 years	24 (70.6)	16 (51.6)	
Patient reported outcome measure			
DRI (median, IQR)	45.2	24.5	0.001
	(0-107.8)	(0-96.1)	

DRI – disability rated index

IM - intramedullary

IQR – Inter quartile range

a: Cases n=34, controls n = 31

Figure 10-4. The proportion of participants who smoke in the 'case' and 'control' groups

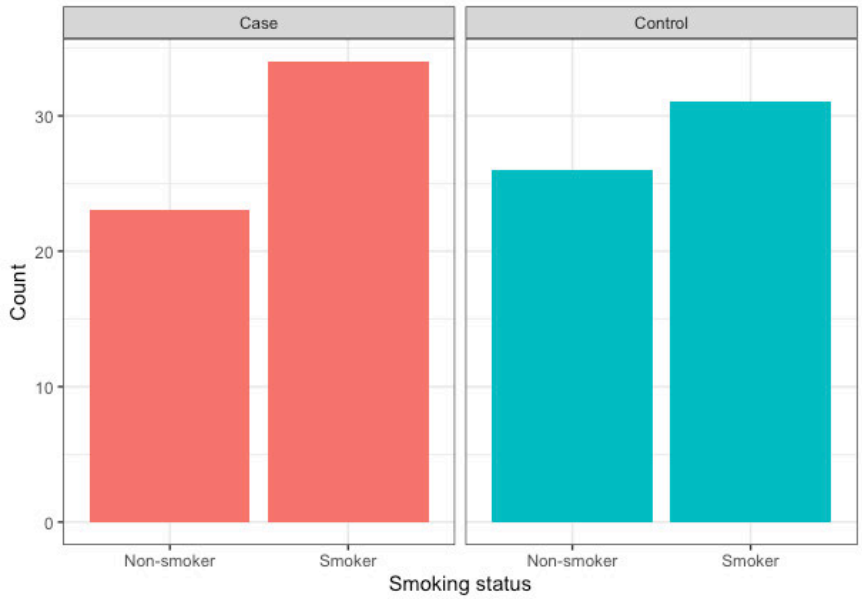
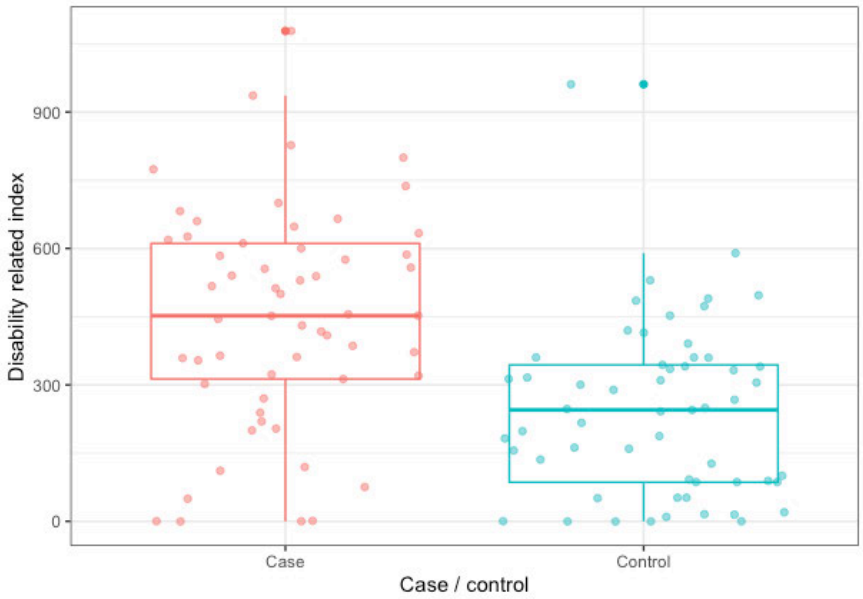


Figure 10-5 The difference in DRI in the ‘case’ and ‘control’ groups. Note that the DRI is a score between 1-100. However, during the data collection process, for the purpose of recording the score, it was recorded in the database as a score between 1-1000 and this is the reason for the difference in the figures on the y-axis.



10.5.3 HIV status

Four of the 'case' participants (7%) had a diagnosis of HIV.(Table 10-6) This is compared to nine participants (15.8%) in the 'control' group, and all of the participants knew about their diagnosis prior to enrolment in the study. Out of the 114 study participants, there were no new diagnoses of HIV on testing at enrolment to the study. Only 50% (2/4) of the cases and 44.4% (4/9) of the controls who were HIV-positive were taking ART and all were taking the same ART regimen (TDF, 3TC + ERV). Although there were only small numbers, the 'control' group of participants had been taking ART for a slightly longer period of time, compared to the cases (1376 days vs 1172 days). The viral load (0.65 vs 2.56 $\log^{10}$ cps/ml) was higher (p-value = 0.3) and the CD4 count (569 vs 393 cell.mm^3) was lower (p-value = 0.2) in the 'control' group, (Figure 10-6) suggesting the participants with HIV in the 'case' group had better control of their HIV virus. However, it is acknowledged that due to the small numbers, it is difficult to draw valid conclusions from this.

Table 10-6 Baseline characteristic of HIV-positive participants

HIV parameter	Cases (Non-union) n=57 (%)	Controls (Union) n=57 (%)	p-value (univariate regression)
HIV status			
Positive	4 (7.0)	9 (15.8)	0.200
Negative	53 (92.0)	48 (84.2)	
Age at time of HIV diagnosis[a] (median, IQR, years)	39.16 (27.25-52.4)	39.24 (27.9-45.21)	1.00
Length of time living with virus[a] (Days: mean)	1237 (758-1854)	736 (176-1655)	0.472
Taking ART on admission[a]			
Yes	3 (75.9)	5 (55.5)	0.502
No	1 (25.0)	4 (44.4)	
Length of time taking ART therapy[b] (Days: mean)	1376 (1242-1450)	1172 (683-1805)	0.450
CD4+ count (cell/mm3)[a] (median, IQR)	569 (520-683)	393 337-610	0.200
Viral load (cps/ml)[a] (log^{10}, median, IQR)	0.65 (0-2.00)	2.56 (1.30-4.68)	0.300

ART – Anti-retroviral therapy

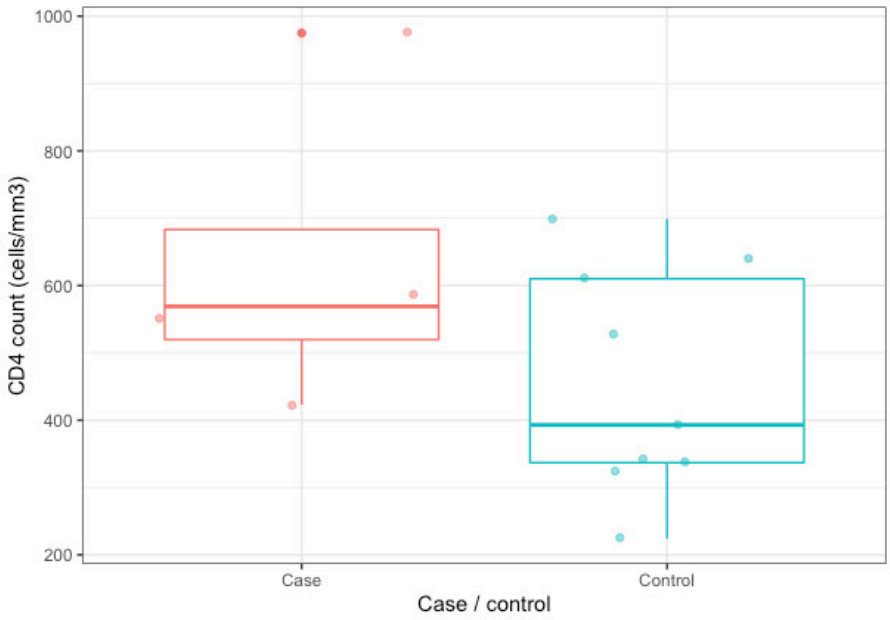
cps – copies

IQR – Inter quartile range

a: n = 4 / 9

b = 3 / 5

Figure 10-6 The CD4 count in the ‘case’ and ‘control’ groups



10.5.4 Medical co-morbidities

A summary of the medical co-morbidities of the two study groups can be seen in Table 10-7. As expected, as a known risk factor for non-union, there were more diabetic participants in the 'case' group, compared to the 'control', although though there was no significant difference(p-value=0.2).(171) There were no other significant differences in the medical risk factors for non-union between the two groups.

Table 10-7 Known medical co-morbidities of study population

Comorbidity	Cases (Non-union) n = 57 (%)	Controls (Union) n = 57 (%)	p-value
Peripheral vascular disease			
Yes	2 (3.6)	0 (0)	0.5
No	55 (96.4)	57 (100.0)	
Rheumatoid arthritis			
Yes	0 (0.0)	0 (0)	1
No	57 (100)	57 (100.0)	
Hypothyroid			
Yes	2 (3.6)	0 (0)	0.5
No	55 (96.5)	57 (100.0)	
Renal impairment			
Yes	0 (0)	0 (0)	1
No	57 (100.0)	57 (100.0)	
Diabetes			
Yes	3 (5.3)	0 (0)	0.2
No	54 (94.7)[a]	57 (100.0)	
Previous Tuberculosis			
Yes	0 (0)	2 (3.6)[b]	0.5
No	57 (100.0)	55 (96.4)	

a. 2 tablets + 1 diet controlled
b. None active and all successfully treated

10.5.5 Household and socioeconomic characteristics

The crowding index was higher in the 'control' group, compared to the cases (1.5 vs 1.25, p-value=0.7). (Table 10-8, Figure 10-7) The crowding index is derived from the total household size divided by the number of living rooms. Higher crowding index has been shown to correlate with socioeconomic status.(415) The 'control' group had a lower number of participants with flushing toilets (51, 89.5% vs 54/57. 94.7%) and piped water (41/57. 71.9% vs 47, 82.5%) to their homes. Furthermore, the 'control' participants had a lower level of completed education. (Table 10-8), overall suggesting that the 'control' participants may originate from a population of lower socioeconomic status. However, the 'case' participants had a higher unemployment rate (31/57, 54.4% vs 24/57, 42.1%) and fewer participants had electricity to their homes (54/57, 94.7 vs 57/57, 100%). Despite these differences in the two groups, none of these were statistically different and no formal socioeconomic score was measured.

Table 10-8. Household and socioeconomic characteristics of study population

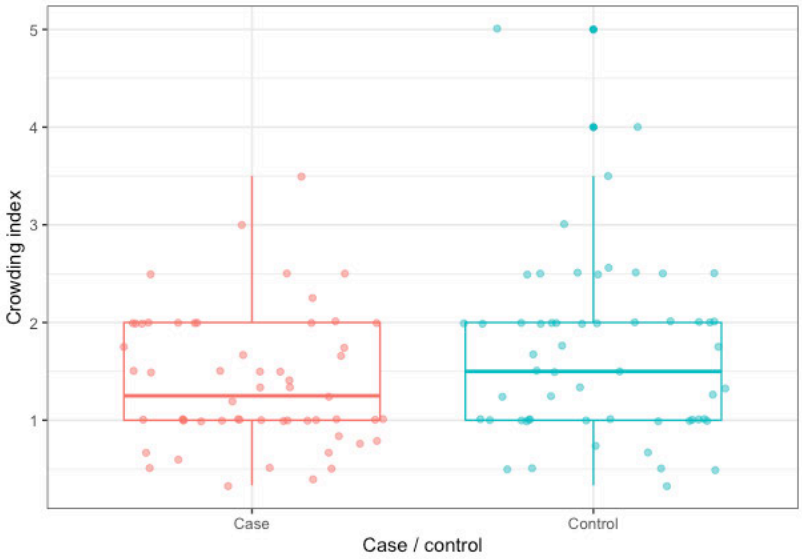
Household and socioeconomic characteristic	**Cases n = 57 (%)**	**Controls n = 57 (%)**	**p-value**
Number of people living in household	4	4	0.4
(median, IQR)	(1-9)	1-18	
Number of living/dwelling rooms in household	3	2	0.3
– kitchen and bathrooms not included	(1-9)	(1-7)	
(median, IQR)			
Crowding index[a] (median, IQR)	1.25	1.5	0.7
	(0.33-3.5)	(0.33-5.0)	
Toilet			
Flush	54 (94.7)	51 (89.5)	0.5
Chemical	0 (0)	0 (0)	
Pit/latrine	1 (1.8)	1 (1.8)	
No sanitation facilities	2 (3.5)	5 (8.7)	
Water supply			
Piped to dwelling	47 (82.5)	41 (71.9)	0.3
Piped to yard	9 (15.8)	15 (26.3)	
Public tap/standpipe	1 (1.8)	1 (1.8)	
Borehole	0 (0)	0	
Principle cooking fuel			
Electricity	54 (94.7)	56 (98.2)	0.5
Gas	2 (3.5)	1 (1.8)	
Wood	0	0	
Coal	0	0	
Other	1 (1.8)	0	
Access to mains electricity to home			
Yes	54 (94.7)	55 (96.5)	1
No	3 (5.3)	2 (3.5)	
Level of education			
Never attended	0 (0)	0 (0)	0.3
Primary	13 (22.8)	10 (17.5)	
Secondary	34 (59.7)	41 (71.9)	
Higher	8 (14.0)	6 (10.5)	
Postgraduate	2 (3.5)	0 (0)	

Employment			
No formal employment (incl. student)	31 (54.4)	24 (42.1)	0.2
Unskilled	18 (31.6)	19 (33.3)	
Skilled	7 (12.3)	14 (24.6)	
Professional	1 (1.8)	0 (0)	

IQR – Inter quartile range

a Total number of household members divided by the number of living rooms in household (kitchen or bathroom not included).

Figure 10-7 The relationship between crowding index in the 'case' and 'control' groups



10.5.6 Blood parameters

The haemoglobin (p-value=0.201), vitamin D (p-value=0.400) and albumin (p-value=0.070) level were all lower in the 'control' group compared to the 'case', although none were statistically significant. (Figure 10-8 , Figure 10-9, Figure 10-10) As discussed in chapter 4, although there is no definite clinical evidence, all three of these blood parameters have been linked to problems of fracture healing and non-union in vivo animal research. (83), (146), (147), (148), (158), (174), (175), A summary of the blood parameter in the two groups can be seen in Table 10-9.

Table 10-9. Blood parameters of study population

	Cases n=57 (%)	**Controls n=57 (%)**	**p-value**
Haemoglobin (g/dL) (median/IQR)	13.2 (6.1-17.3)	9.8 (5.9- 15.9)	0.201
White blood cells (x 10^9 / L) (median/IQR)	7.36 (3.5-14.4)	8.7 (3.2-39.1)	0.400
Creatine[a] (umol/L) (median/IQR)	64 (40-106)	61 (31-94)	0.400
Urea[b] (mmol/L) (median/IQR)	4 (1.7-9)	3.9 (1.2-6.6)	0.501
Vitamin D[c] (nmol/L) (median/IQR)	59.65 (21.4-110.2)	50.35 (18 – 89.5)	0.402
Albumin[d] (g/L) (median/IQR)	44.5 (22-51)	35 (20-49)	0.070

a: n=57 / 55

b: n= 57 / 55

c: n= 56 / 52

d: n= 56 / 52

Figure 10-8 The haemoglobin level of the ‘case’ and ‘control’ groups

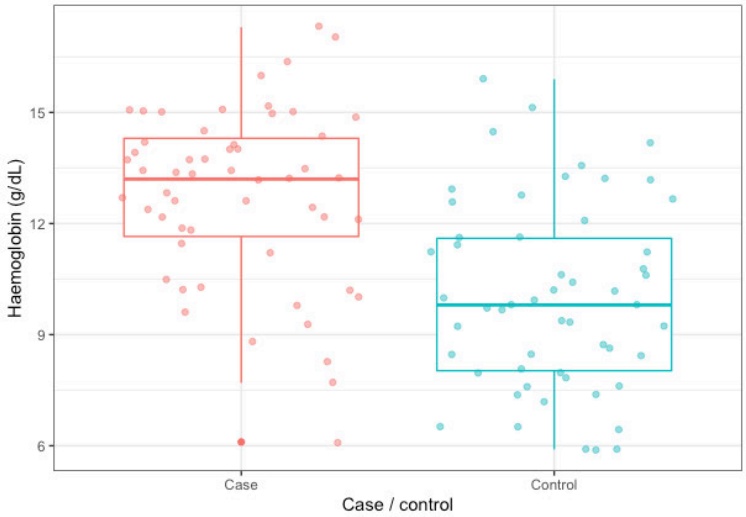


Figure 10-9 The vitamin D level of the ‘case’ and ‘control’ groups

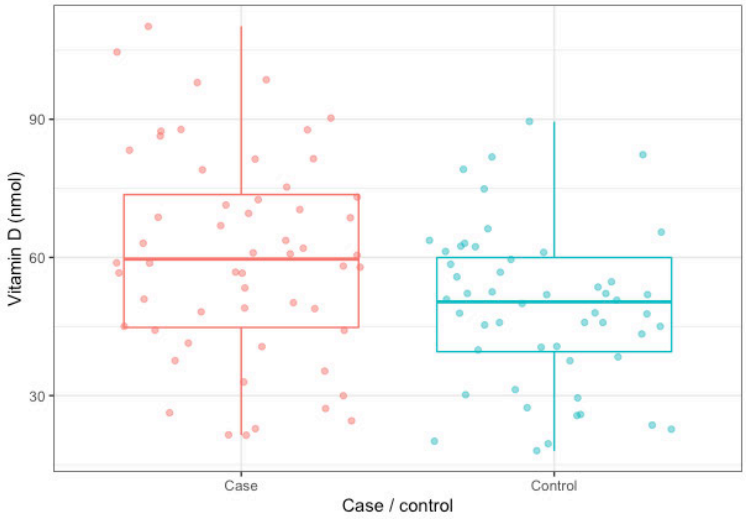
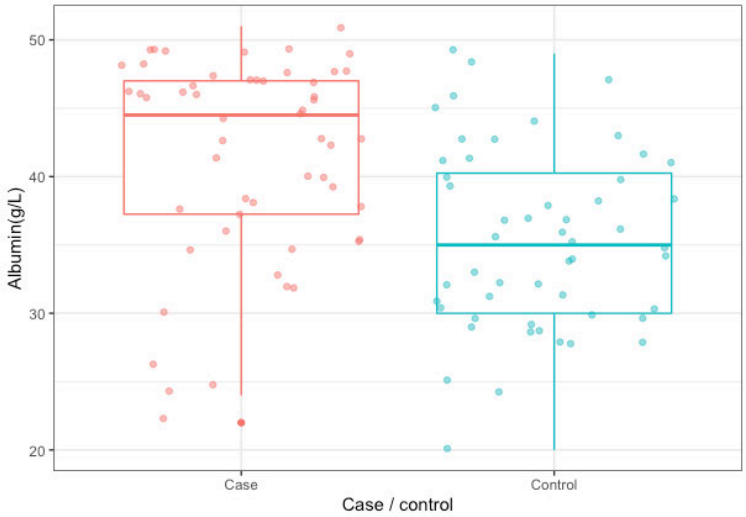


Figure 10-10 The albumin level of the ‘case’ and ‘control’ groups



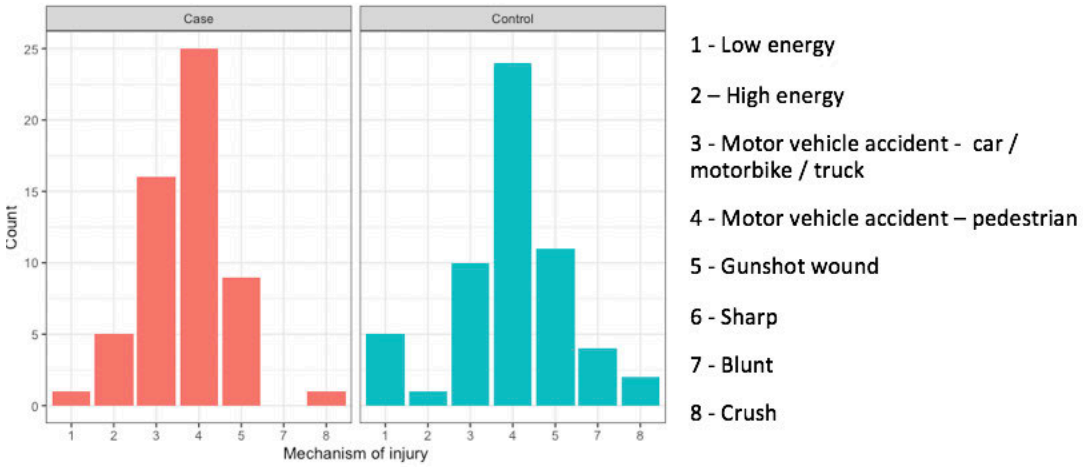
10.5.7 Mechanism of injury

Fifty-five out of 57 (94.7%) cases were due to injuries that resulted from high energy mechanisms (5/57), motor vehicle accidents (41/57) and GSWs (9/57).(Table 10-10, Figure 10-11) This is compared to only 46/57 (80.7%) in the control group. This echoes the current evidence in the literature suggesting that higher energy injuries result in an increase in the non-union rate following a fracture, although this difference was not statistically significant (p-value=0.08). (190), (189) A summary of the mechanism of injury for the two study groups can be seen in Table 10-10.

Table 10-10. The mechanism of injury of study population

Mechanism of injury	Cases n=57 (%)	Controls n=57 (%)	p-value
Low energy	1 (1.8)	5 (8.8)	0.080
High energy	5 (8.8)	1 (1.8)	
Motor vehicle accident - car / motorbike / truck	16 (28.1)	10 (17.5)	
Motor vehicle accident – pedestrian	25 (43.9)	24 (42.1)	
Gunshot wound	9 (15.8)	11 (19.3)	
Low energy	9 (100.0)	10 (90.9)	
Medium energy	0	1 (9.1)	
High energy	0	0	
Blunt	0	4 (7.0)	
Crush	1 (1.8)	2 (3.5)	

Figure 10-11 The mechanism of injury of the study participants in the ‘case’ and ‘control’ groups



10.5.8 Open fracture and additional injuries

There was a higher proportion of open fractures in the 'control' group compared to the cases (28/57, 49.1% vs 22/57, 38.6%, p-value=0.300).(Figure 10-12) Additionally, more participants in the 'control' group had additional injuries as well as their fracture at the time of injury (1/57, 26.3 vs 11/57, 19.3%, p-value=0.500) and had a higher injury severity score (IIS) (15/57, 26.3% vs 13/57, 22.8%, p-value=0.800).

The higher the GA grade were more likely to be managed with a circular fixator. Therefore, there were more circular fixators used in the 'control' group when compared to the cases. (Table 10-12)

A summary of the number of open fractures, additional injuries, summary of fixation methods and IIS for the two study groups can be seen in Table 10-11 and Table 10-12.

Table 10-11. Open fractures and additional injury parameters of study population

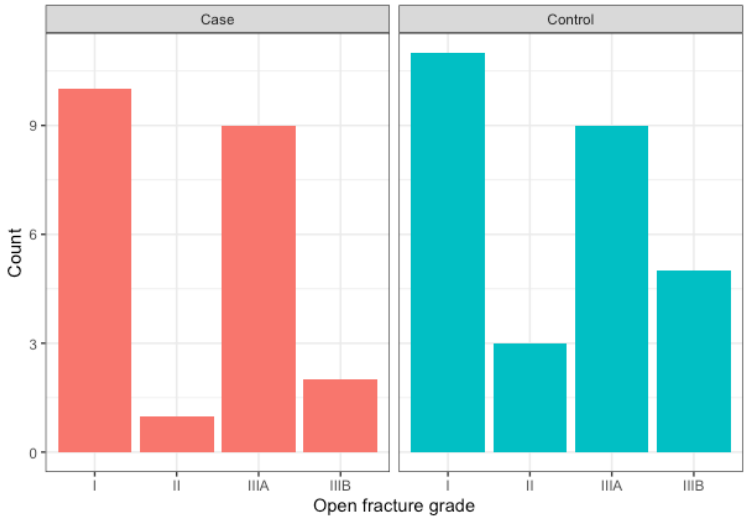
	Cases n=57 (%)	Controls n=57 (%)	p-value
Open fracture			
Yes	22 (38.6)	28 (49.1)	0.300
No	35 (61.4)	29 (50.9)	
Gustilo Anderson Classification [a]			
I	10 (45.5)	11 (39.3)	0.700
II	1 (4.5)	3 (10.7)	
IIIA	9 (40.9)	9 (32.1)	
IIIB	2 (9.1)	5 (17.9)	
IIIC	0 (0)	0 (0)	
Additional injuries			
Yes	11 (19.3)	15 (26.3)	0.500
No	46 (80.7)	42 (73.7)	
Injury severity score ≥ 16			
Yes	13 (22.8)	15 (26.3)	0.800
No	44 (77.2)	42 (73.7)	

a: n=22 / 28

Table 10-12. A summary of the methods of fixation of open fractures according to Gustilo Anderson Grade

CASES n = 22	Conservative plaster n = 1 (%)	IM nailing n = 12 (%)	Plate and screws n = 0 (%)	Circular frame n = 9 (%)
Gustilo Anderson Classification				
I	1 (4.5)	7 (31.8)	0 (0)	2 (9.1)
II	0 (0)	1 (4.5)	0 (0)	0 (0)
IIIA	0 (0)	3 (13.6)	0 (0)	6 (27.3)
IIIB	0 (0)	1 (4.5)	0 (0)	1 (4.5)
IIIC	0 (0)	.0 (0)	0 (0)	0 (0)
CONTROLS n = 28	**Conservative plaster n = 0 (%)**	**IM nailing n = 15 (%)**	**Plate and screws n = 0 (%)**	**Circular frame n = 13 (%)**
Gustilo Anderson Classification				
I	0 (0)	9 (32.1)	0 (0)	2 (7.1)
II	0 (0)	3 (10.7)	0 (0)	0 (0)
IIIA	0 (0)	2 (7.1)	0 (0)	7 (25.0)
IIIB	0 (0)	1 (3.6)	0 (0)	4 (14.3)
IIIC	0 (0)	.0 (0.0)	0 (0)	0 (0)

Figure 10-12 The Gustilo Anderson open fracture grade of the ‘case’ and ‘control’ groups



10.5.9 Classification and fracture pattern

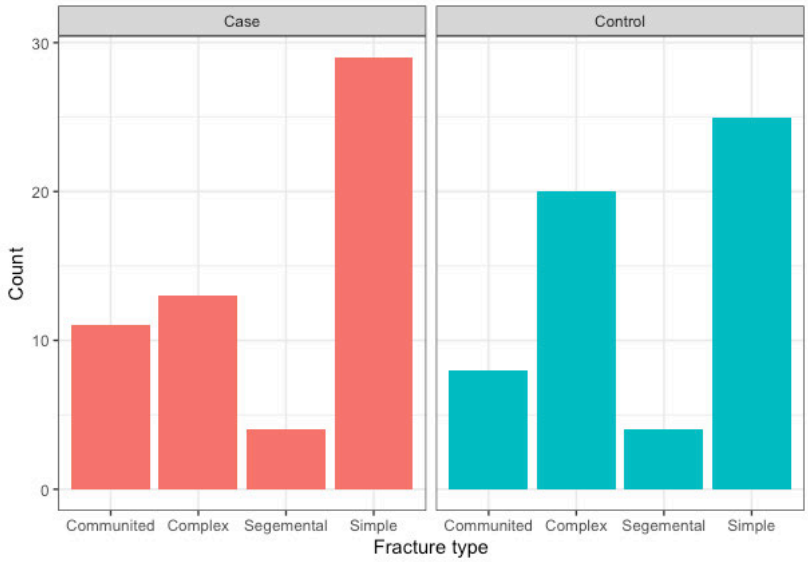
It has been established that fractures that are unstable and comminuted are associated with problems of fracture healing. (193), (198), (404) However, it was the 'control' study group that had a higher proportion of fractures that were classified as unstable or highly unstable compared to the 'case' study group using the AO classification system (Appendix 13-8) (28/57, 49.1% vs 24/57, 42.1%, p-value=0.6). Although these differences were not statistically different. (Figure 10-13) A summary of the number of fracture classification and x-ray parameters for the two study groups can be seen in Table 10-13.

Table 10-13 Fracture classification and pattern of study population

	Cases n=57 (%)	Controls n=57 (%)	p-value
Simple – stable			
A2	11 (19.3)	4 (7.0)	0.600
A3	11 (19.3)	9 (15.8)	
B2	7 (12.3)	12 (21.1)	
Total	29 (50.9)	25 (43.9)	
Complex - unstable			
A1	2 (3.5)	9 (15.8)	
B1	4 (7.0)	8 (14.0)	
B3	7 (12.3)	3 (5.3)	
Total	13 (22.8)	20 (35.1)	
Comminuted - highly unstable			
C1	4 (7.0)	1 (1.8)	
C3	7 (12.3)	7 (12.3)	
Total	11 (19.3)	8 (14.0)	
Segmental - potentially unstable			
C2	4 (7.0)	4 (7.0)	
Total	4 (7.0)	4 (7.0)	
Winquist classification			0.300
Femur (n=20)			
Type 0	4 (20.0)	4 (20.0)	
Type 1	3 (15.0)	3 (15.0)	
Type 2	6 (30.0)	6 (30.0)	
Type 3	3 (15.0)	3 (15.0)	
Type 4	4 (20.0)	4 (20.0)	

IQR – Inter quartile range

Figure 10-13 The AO fracture classification of the ‘case’ and ‘control’ study groups



10.5.10 Time between enrolment and injury

Cases were enrolled following the development of a non-union. By definition this is after at least 9 months from their injury, whereas the controls were enrolled during the fracture healing phase or at the end of fracture healing. Therefore, the length of time between a participant's injury and enrolment in the HOST 2 study is likely to be longer for the cases. Table 10-14 confirms the time between the date of participants injury and enrolment into the study was longer for the cases.

Table 10-14. Summary of the length of time in days, between injury and baseline enrolment

	Case **n = 57**	**Control** **n = 57**
Time injury and enrolment (Days: median: IQR)	320 (276-523)	180 (122-241)

CI – Confidence interval

IQR – Interquartile range

10.5.11 Type of non-union

Non-unions were categorised into two main types: hypertrophic, (the primary factors influencing non-union is mechanical) and atrophic/oligotrophic, (the primary factor influencing non-union is local fracture and/or systemic biology).(210)

There were 32/57 (56.1%) atrophic and 25/57 (43.9%) hypertrophic non-unions in the 'case' study cohort. Of the four participants who were HIV-positive three (3/4, 75%) had atrophic non-unions. The majority of non-unions that developed were following IM nailing (36/57, 63.2%), with 71.9% (23/36) of IM nailings non-unions resulting in atrophic non-unions in this study populations (p-value=0.08).(Figure 10-14)

A higher crowding index higher (1.4 vs 1, p-value=0.500) was seen in the in atrophic non-union subgroup, as was a lower albumin level (40.5 vs 46 g/L, p-value=0.800). (Figure 10-15, Figure 10-16) However, although both suggest nutritional and socioeconomic status may have an association with atrophic non-unions in this study cohort, with such small numbers and no formal validated assessment of socioeconomic status made, it is difficult to draw any valid conclusion and none were statistically significant.

Open fractures (14/32, 43.7% vs 8/25, 32%, p-value=0.500), pedestrians hit by motor vehicle (15/32, 46.9% vs 10/25, 40.0%) and GSW fractures (6/32, 18.8% vs 3/25, 12%) resulted in a higher proportion of atrophic than hypertrophic non-unions. As discussed in previous chapters, open fractures and high energy mechanism of injury are associated with periosteal stripping and potential local vascular problems at the fracture site. To add further to this, atrophic non-unions were more unstable (17/32, 53.1% vs 7/25, 28.0%, p-value=0.200), compared to hypertrophic non-unions. (Figure 10-17)

A summary of the number of the non-union types and baseline parameters for the two study groups can be seen in Table 10-15.

Table 10-15 Type of non-union in study population

	Atrophic n = 32 (%)	Hypertrophic n = 25(%)	p-value
Type of non-union	32 (56.1)	25 (43.9)	
HIV-positive	3 (5.3)	1 (1.8)	0.100
ART therapy	3 (6.5)	1 (4)	1
Management			
Plaster	2 (6.3)	1 (4.0)	0.080
IM nailing	23 (71.9)	13 (52.0)	
Plate and screw fixation	1 (3.1)	1 (4.0)	
Circular fixator	6 (18.8)	10 (40.0)	
Crowding index	1.4 (0.4-3.5)	1 (0.33 – 2)	0.500
Haemoglobin	13.2 (6.1-17.3)	13.55 (9.8-17.3)	0.400
(median: IQR, g/dL)			
Vitamin D	59.65 (21.4-	57.25 (24.5-	0.900
(median: IQR, nmol)	110.2)	110.2)	
Albumin (g/L)	40.5 (24-49)	46 (22-51)	0.800
Mechanism of injury			
Low energy	0	1 (4.0)	0.500
High energy	3 (9.4)	2 (8.0)	
Motor vehicle accident - car/motorbike/truck	7 (21.9)	9 (36.0)	
Motor vehicle accident – pedestrian	15 (46.9)	10 (40.0)	
Gunshot wound	6 (18.8)	3 (12.0)	
Crush	1 (3.1)	0	
Open fracture			
Yes	14 (43.7)	8 (32.0)	0.500
No	18 (56.3)	17 (68.0)	
Classification - type (n=14 / 8)			
I	8 (57.1)	2 (25.0)	0.300
II	1 (7.1)	0 (0)	
IIIA	4 (28.6))	5 (62.5)	
IIIB	1 (7.1)	1 (12.5))	
IIIC	0 (0)	0 (0)	

Classification			
Simple	12 (37.5)	17 (68.0)	0.2
Complex - unstable	9 (28.1)	4 (16.0)	
Comminuted - highly unstable	8 (25.0)	3 (12.0)	
Segmental	3 (9.4)	1 (4.0)	

IQR – Inter quartile range

Figure 10-14 The fracture management of the 'case' participants according to non-union type

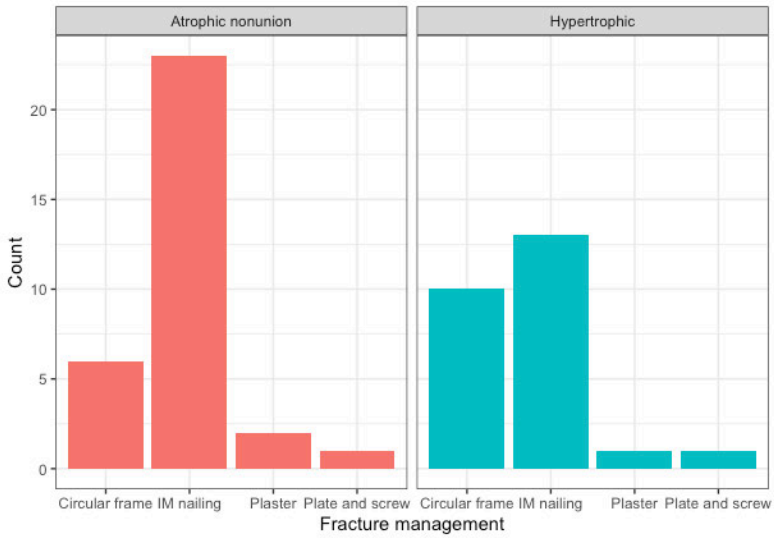


Figure 10-15 The crowding index of the ‘case’ participants according to non-union type

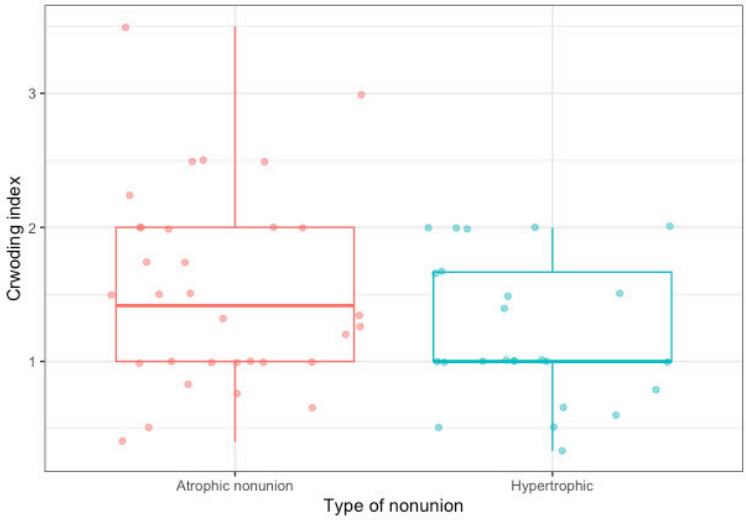


Figure 10-16 The albumin of the ‘case’ participants according to non-union type

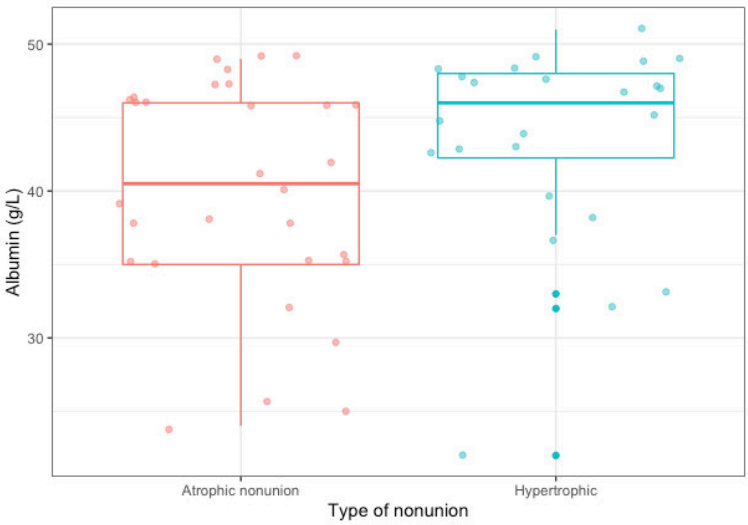
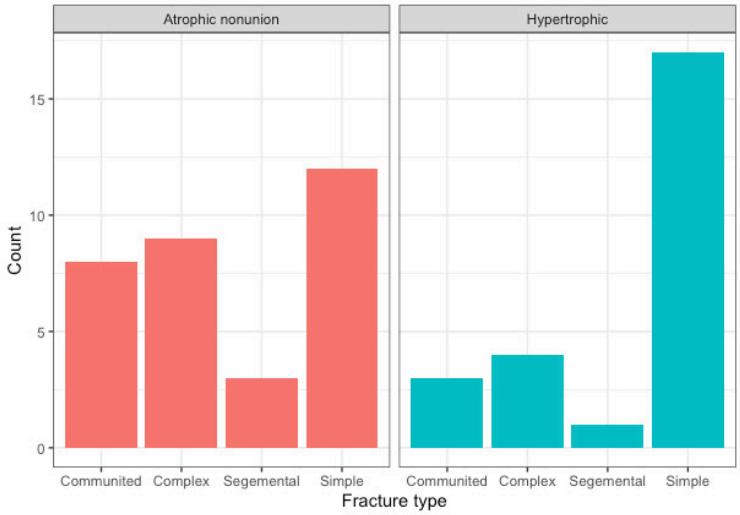


Figure 10-17 The fracture classification of the ‘case’ participants according to non-union type



CHAPTER 11. HIV IN ORTHOPAEDIC SKELTAL TRAUMA 2 STUDY 2: Analysis of the Primary and Secondary Outcomes and Discussion

11.1 Aims of this chapter

This chapter details the analysis of the primary and secondary outcomes of the HOST 2 Study and ends with a discussion regarding the study.

11.2 Results

11.2.1 Final study population

The main study cohort of 57 non-union cases were matched with 57 union 'control' participants. The primary outcome of fracture non-union was defined as one or both of the following:

- Impaired bone healing at nine months on RUST score (RUST score < 9). (125), (126), (127), (128)

Parameters and confounding factors included in the univariate and multivariable logistic regression model were:

1. HIV status
2. Age
3. Sex
4. Fracture management
5. Fracture site
6. Smoking status
7. Open fracture
8. Haemoglobin at baseline
9. Vitamin D at baseline

Of the 57 non-unions cases, 7.0% (4/57) occurred among HIV-positive participants, compared to 15.7% (9/57) in the controls. On univariate and multivariable logistic regression analysis, HIV was not statistically associated with the development of a non-union in the study population (univariate OR 0.40 [CI 0.10-1.32] p-value = 0.151, multivariable OR 0.85 [0.18-3.73] p -value = 0.831).

The haemoglobin level was lower in the 'control' group (median 9.8 per 1 g/dL [8.02-11.6]) participants than in the cases (median 13.2 per 1 g/dL [11.6-14.3]). The univariate (OR 1.55 [CI 1.31-1.89], p-value = 0.001) and multivariable analysis (OR 1.64 [CI 1.33-2.09] p-value = 0.001) confirmed that the higher the level of haemoglobin, the more likely a participant was to have a non-union, both of which were statistically significant.

Vitamin D level was lower in controls (median 50.4 per 1 nmol/L (39.5-60.0)) participants than in the cases (median 59.65 per 1 nmol/L (44.8-73.6)) The univariate analysis, (1.03 [1.01-1.05] p-value = 0.005) showed that the higher the level of vitamin D in the study population, the more likely a participant was to have a non-union, but on multivariable analysis although this increase in risk of non-union was shown, it was not statistically significant (1.02 [1.00-1.05], p-value = 0.069).

Age, sex or smoking were not shown to be associated with the development of non-union on both univariate and multivariable analysis (Table 11-1). A summary of the univariate and multivariable logistic regression model analysis can be found in Table 11-1.

Table 11-1. Risk factors for the development of non-union in the study population

	Cases – Non-union n=57, %	Controls – union n = 57, %	Univariate odds ratio (95% CI)	p-value	Multivariable odds ratio (95% CI)	p-value
HIV status						
HIV-negative	53 (92.0)	48 (84.2)				
HIV-positive	4 (7.0)	9 (15.8)	0.40 (0.10-1.32)	0.151	0.85 (0.18-3.73)	0.831
Age (per year)	36 (21-76)	36 (18-63)	1.01 (0.98-1.04)	0.530	1.01 (0.97-1.06)	0.593
Sex						
Male	44 (77.2)	44 (77.2)				
Female	13 (22.8)	13 (22.8)	1.00 (0.41-2.41)	1.000	2.36 (0.74-8.09)	0.155
Fracture management						
Plaster	3 (5.3)	3 (5.3)	1.00 (0.792-1.26)	1.000	1.21 (0.89-1.66)	0.236
IM nailing	36 (63.2)	36 (63.2)				
Plate + screws	2 (3.5)	2 (3.5)				
Circular fixator	16 (28.1)	16 (28.1)				
Fracture site						
Femur	37 (63.2)	37 (63.2)				
Tibia	20 (36.8)	20 (36.8)	1.00 (0.43-2.16)	1.00	0.348 (0.10-1.05)	0.071
Smoking status	20 (36.8)	20 (36.8)				
Yes	23 (40.4)	26 (45.6)	1.24 (0.59-2.62)	0.577	1.24 (0.47-3.30)	0.662
No	34 (59.6)	31 (54.4)				
Open fracture						
Yes	22 (38.6)	28 (49.1)	0.65 (0.31-1.37)	0.258	1.21 (0.43-3.57)	0.716
No	35 (61.4)	29 (50.9)				

Haemoglobin (IQR, per 1 g/dL)	13.2 (11.6-14.3)	9.8 (8.02-11.6)	1.55 (1.31-1.89)	0.001	1.64 (1.33-2.09)	0.001
Vitamin D (IQR, per 1 nmol/L)	59.65 (44.8-73.6)	50.4 (39.5-60.0)	1.03 (1.01-1.05)	0.005	1.02 (1.00-1.05)	0.069

CI – Confidence intervals

IQR – Interquartile range

11.2.2 Determining union and non-union using the RUST score – inter-observer reliability.

Two independent reviewers (reviewer 1 and 2), blinded to the HIV status of the participant, reviewed all x-rays and determined union (controls) and non-union (cases) using the RUST score for the femur or tibia fracture. On four x-rays (4/114), in four participants, there was a lack of consensus between the two reviewers on the outcome. Therefore, a third reviewer was used to determine the outcome. The third reviewer (reviewer 3) was again blinded to the HIV status of the participant and also the score the two reviewers had given for the x-ray they were reviewing. Out of the four disagreements, two were in cases participants and two were in a 'control'.

The inter-observer agreement, between reviewers 1 and 2, of the outcome of union or non-union using the final RUST score was 96.5% (Kappa = 0.93).

The reviewers also determined the type of non-union a participant had developed (hypertrophic or atrophic/oligotrophic). The inter-observer agreement between the two reviewers was 100% (Kappa 1).

11.2.3 Non-union in the HOST 1 and HOST 2 studies

When combining the HIV-positive participants from the HOST 1 and HOST 2 studies there was a total of 96 fractures in 84 HIV-positive participants. Overall there were five non-unions that were HIV-positive all together (5.2%, 5/96), four of which were taking ART therapy (80%, 4/5).

Note that as part of the HOST 2 study methodology a number of the non-unions and unions from HOST 1 were included in the HOST 2 study as cases. The HIV-positive participant from the HOST 1 study developed a non-union after recruitment for the HOST 2 study had closed. Therefore, this participant was not included in the final HOST 2 study population. Seven HIV-positive participants from the HOST 1 were included in the HOST 2 study as controls. When combining the data, these seven participants were removed. Therefore, there were 89 fractures in 77 HIV-positive participants included in this analysis.

When analysing the CD4 count of the participants, the HIV-positive participants whose fractures had united had a mean CD4 count that was higher than that of the participants who developed non-union, in both ART naïve and participants taking ART. (Table 11-2, Figure 11-1. The *CD4 count at baseline in the two studies combined stratified for outcome and ART regimen.*

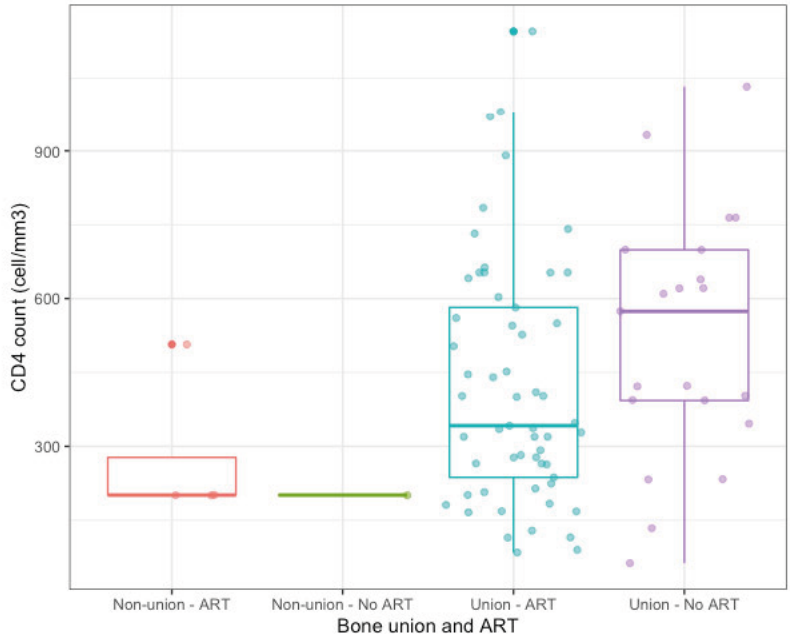
Table 11-2. The CD4 count of participants according to the participants outcome and ART therapy

	Baseline			
	Union – ART n = 60	**Union – No ART n = 24**	**Non-union ART n = 4**	**Non-union no ART n = 1**
CD4 count cell mm3 (Mean: IQR)	420 (244-577)	522 (393-699)	278 (201-278)	201

IQR – interquartile range

Means have been reported due to the low number of participants in the non-union groups.

Figure 11-1. The CD4 count at baseline in the two studies combined stratified for outcome and ART regimen.



11.3 Discussion – HOST 2 study

11.3.1 Principal findings

The HIV in Orthopaedic Skeletal Trauma Study 2 showed that HIV status was not associated with the development of non-union following the management of tibia and femur fractures, on both univariate (OR 0.40 [0.10-1.32], p-value = 0.151) or multivariable (OR 0.86 [0.18-3.73], p-value = 0.831) logistic regression analysis. Higher levels of post-operative vitamin D and haemoglobin were shown to increase the odds of non-union on univariate analysis. However, on multivariable analysis of vitamin D level, this difference was found not to be statistically significant (OR 1.02 [1.00-1.05] p-value = 0.069). However, the haemoglobin finding remained statistically significant on multivariable analysis (OR 1.64 [CI 1.33-2.09] p-value = 0.001). No other confounding factors was not shown to have any statistically significant impact on the odds of developing non-union in this study cohort, including open fracture and smoking.

11.3.2 HIV status in study population

The prevalence of HIV in the case was 7% (4/57) and 15.8% (9/57) in the controls. There was an overall prevalence of 11.4% (13/114) in the study population. The prevalence of HIV in the HOST 1 study population was 19.8% (71/358 participants) and the national prevalence of HIV in South Africa is approximately 18.9%.(57), (58) As discussed previously, the prevalence in the Western Cape is much lower at 5.6%.(57), (58) The overall prevalence in the HOST 2 study was therefore over double the region rate and although the prevalence appears to be low when compared to the HOST 1 study, this study population does in fact represent a cohort of the population with a higher prevalence of HIV compared to the regional average. 77.2 % (44/57) of the participants were male, similar to the finding from the HOST 1 study,

that this study population are more likely to suffer a traumatic injury and have higher rates of HIV than the national average.

Seventy-five percent (3/4) of the cases and 44.4% (4/9) of the controls who were HIV-positive were taking ART. This is slightly higher than those in the HOST 1 study (69 % [49/71]) and the national average (61%). (53) The low number of participants make firm conclusions difficult, but this suggests that a higher proportion of participants on ART develop a non-union.

The study sample size calculation anticipated a prevalence of 20% of HIV in the 'control' group. Overall, the rates of HIV in both study 'case' and 'control' groups were lower than anticipated, as a result any conclusions need to be interpreted with caution.

11.3.3 Comparison with the published literature

There have been no previously published case-control studies in the literature investigating HIV and fracture healing. As previously discussed in the HOST 1 study discussion, the literature to date has reported overall non-union rate of between 0-11% (213), (367), (382), (389), (394), (439) in HIV-positive individuals following numerous different methods of fracture fixation. These reports cannot be compared to the outcome of this study due to the differences in study design.

11.3.4 Parameters that influence the study outcome

Socioeconomic factors

The relationship between social deprivation and fracture healing was discussed in the HOST 1 study. In summary, there is no reliable evidence to confirm socioeconomic status as clear risk factor for the development of delayed or non-union or infection following fracture fixation.(454)

The crowding index was slightly higher in the 'control' group compared to the cases (1.5 vs 1.25, p-value=0.7). The 'control' group had a lower number of participants with flushing toilets (51, 89.5% vs 54/57, 94.7%) and piped water (41/57, 71.9% vs 47, 82.5%) to their homes. Furthermore, the 'control' participants had a lower level of completed education. Therefore, although no parameter was shown to be statistically different between the two groups, the 'control' participants may originate from a population of lower socioeconomic status compared to the 'case' group, but this is unlikely to have influenced the study outcomes.

Smoking status

There is strong evidence demonstrating the link between smoking, particularly nicotine, to problems with fracture healing.(163) However, in this study population, smoking was not found to be associated with the development of non-union on multivariable analysis (OR 1.24 [CI 0.47-3.30) p-value = 0.662) There were similar proportions of smokers in both the 'case' (23/57 40.4%) and 'control' (26/57, 45.6%) groups. This mirrored the findings from the HOST 1 study, were delayed union (multivariable OR 0.414 [CI 0.17-1.01] p-value = 0.113) and non-union (multivariable OR 1.67 [CI 0.68-4.67] p-value = 0.303) was not higher in those participants that smoked on both univariate and multivariable analysis.

The current prevalence of smoking South Africa is approximately 17.6%. It has been reported that males (29.2%) have a prevalence four times higher than that of females (7.3%) (OR 5.20 [CI 4.39 - 6.16] p-value = 0.001).(467) The prevalence in this study population is 43.0% (49/114), more than double the population average. This again highlights the potential high-risk taking behaviour of the study population group. The high smoking proportion in each group does not explain why, unlike in the established literature smoking was not shown to be a factor associated with the development of non-union in both studies.

Blood parameters

The haemoglobin (9.8 vs 13.2 g/dl, p-value=0.2), vitamin D (50.35 vs 59.65 nmol/L, p-value=0.4) and albumin (35 vs 44.5 g/L, p-value=0.07) levels were all lower in the 'control' compared to the cases, although none of the differences between the groups were statistically significant. All three of these blood parameters have been linked to problems of fracture healing and non-union in in vivo animal research.(146), (83), (147), (148), (174), (175), (158) Therefore, these findings are contrary to what would have been expected, with lower levels of haemoglobin, vitamin D and albumin anticipated in the cases.

Univariate (OR 1.55 (CI 1.31-1.89), p-value = 0.001) and multivariable (OR 1.64 [CI 1.33-2.09] p-value = 0.001) logistic regression analysis of the study cohort confirmed that the higher the level of haemoglobin a participant had, the more likely they were to have a non-union. This may be explained by the difference in the time between the date of injury and enrolment into the study for the cases compared to the controls (320 days [276-523] vs 180 [122-241] p-value = 0.001). cases had longer to recover from their injury and any surgery, potentially leading higher haemoglobin levels.

Any effect on fracture healings that may result from lower levels of vitamin D, albumin and haemoglobin are likely to only be evident at the extreme ends of the

values for each blood parameter. For example, as discussed in chapter 9, vitamin D level in the cases and controls was within the 'normal' range and not < 25 nmol/L which is used by the National Institute for Health and Care Excellence as a definition of vitamin D deficiency. (448) Therefore, essentially, the values of vitamin D were very similar between each group and of limited clinical significance.

Open fractures

There was a higher proportion of open fractures in the 'control' group compared to the cases (28/57, 49.1% vs 22/57, 38.6%, p-value=0.3). It would be expected that the number of open fractures would be higher in the 'case' group, rather than the 'control', since there is established evidence that open fractures have a higher rate of non-union. (210), (456), (457) Contrary to the HOST 1 study and published literature, open fractures were not shown to be a statistically significant risk factor for the development of non-union in the study population.

This cannot be explained by the high number of GSWs in this study population as there was a similar proportion of GSWs in each of the two groups. Nine of the 22 (40.9%) and eleven of 28 (39.3%) open fractures were GSW fractures in the 'case' and 'control' group respectively. GSWs are open fractures by definition but the pathogenesis of how the fractures heal and outcomes are different from a GA grader II and above open fracture.(404), (468)

The proportion of fractures that progressed to union following an open fracture in the HOST 1 study population was 32.8% (122/372). This is lower than the number in the 'control' group in this study (49.1%, 28/57). The proportion of non-unions that resulted from open fractures in the HOST 1 study was 65% (15/23), compared to 38.6% (22/57) in this study.

It may, therefore, be a combination of the number of open fractures in the 'case' group being slightly lower than expected and the number of open fractures in the

'control' group being slightly higher than expected, due to the inclusion of GSWs as open fractures, that resulted in open fractures not being found to be a risk factor for non-union in this study populations.

Conversely in the HOST 1 study, 54.7% (75/137, 54.7%) of open fractures were GSW and having an open fracture was shown to have a higher proportion of non-unions. In conclusion, it is not clear why open fractures were not shown to be a risk factor for the development of non-union in this study population, but it is likely to be the result of a small number of study participants.

11.3.5 Limitations of this work

One of the main limitations of this study is the selection of 'control' participants. Once a participant was enrolled as a 'case', the study team commenced recruitment to identify an appropriate 'control' participant using a number of methods previously discussed in the methodology part of this thesis. During this process, no formal blinding to HIV status was undertaken and therefore this has the potential to introduce bias into the selection process. Furthermore, the participants were only matched for age, sex, management and fracture site. They were not matched for parameters such as day of the week admitted, day operated on or the operating surgeon. This was logistically not possible; however, it is acknowledged as limitation of the study.

The initial sample size calculation, assumed that 20% of the controls would be HIV-positive (368), (388), and therefore a total sample size of 128 (64 cases and 64 controls) would give 80% power to detect at least an odds ratio of 3.0 for non-union, comparing between the case and controls groups. The prevalence of HIV was much lower (11.4% [13/114]) than 20% in the study population and therefore the study was underpowered when interpreting outcomes.

The study included all participants who had developed a non-union, irrespective of the type of non-union that developed or the method of management for the fracture. Ideally, all cases should have been managed with one method of fixation and only one type of non-union included to limit the introduction of confounding factors.(116)

The presence of established infection at the fracture site was an exclusion criterion. However, in a high proportion of non-unions, up to 40% have undiagnosed underlying infection.(210) If the fractured limb appeared infection-free on inspection, no microbiology assessment was included in the diagnosis of non-union. No intra-operative microbiology samples following non-union surgery were assessed. Therefore, some of the cases included could have been undiagnosed infected non-unions.

Six participants were not matched with cases and therefore were excluded from the study and not included in the analysis. None of these participants were HIV-positive, but it is recognised that this could have introduced bias in the selection process, and ideally, matches should have been found.

11.3.6 Summary

This is the first case-control study ever performed investigating non-union of a fracture in HIV-positive individuals. HIV was shown not to be associated with the development non-union following the management of the tibia or femur in our study population.

CHAPTER 12. OVERALL DISCUSSION

12.1.1 Aims of chapters

In this chapter a summary of the findings of this thesis will be discussed, and further details of the strengths and weaknesses of the work performed. Any health policy implications will be explored and suggestions for future work will be considered.

12.1.2 Principal findings of the research

The primary hypothesis of this research was that HIV causes delayed bone union and is a risk factor for the development of non-union following a fracture.

12.1.2.1 Delayed union

This research demonstrates that HIV is not associated with the development of delayed union following an IM nailing for a fracture to the tibia or femur. (univariate OR 0.76, [CI 0.37-1.44], p-value=0.417, multivariable OR 1.06 [CI 0.50-2.22], p-value=0.869).

12.1.2.2 Non-union

The proportion of non-unions was statistically significantly lower in HIV-positive participants in the HOST 1 study, compared to HIV-negative participants. (univariate OR 0.16 [CI 0.01-0.78], p-value = 0.076, multivariable OR 0.17 [CI 0.01-0.92], p-value = 0.100) The HOST 2 further demonstrated no evidence of association between HIV status and the development of non-union, between cases and controls. (univariate OR 0.40 [CI 0.10-1.32] p-value = 0.151, multivariable OR 0.85 [0.18-3.73] p -value = 0.831). In summary, HIV is not a risk factor for the development of non-union in either the HOST 1 or HOST 2 studies and fracture healing may be improved in HIV-positive participants, with lower rates of non-union.

12.1.3 Secondary findings of research

12.1.3.1 Delayed union

In HIV-positive participants, there was no association between delayed union and the level of CD4 count (multivariable OR 1.00 [CI 0.97-1.00] p-value = 0.181) or viral load (multivariable OR 1.03 [CI 0.66-1.58] p-value = 0.897). 57.7% (41/71) of participants in the HOST 1 study were taking ART on enrolment. This increased to 69.0% (49/71) at six-month follow-up. The proportion of delayed union in ART naïve participants was higher at 23.3% (7/30), compared to 12.2% (5/41) in those participants taking ART on enrolment. However, the true effect of ART on delayed fracture union cannot be determined due to the low number of participants taking ART taking at enrolment (41/71).

12.1.3.2 Non-union

The number of participants who developed non-union and were HIV-positive in both studies was low, making it difficult to draw any valid conclusions from this data. On combining non-union data from the HOST 1 and HOST 2 study, 80% (4/5) of the HIV-positive participants who developed non-union were taking ART on enrolment. This is in comparison with 56% of HIV-positive participants who were taking ART and went on to fracture union when combining the HOST 1 and 2 studies data (HOST 1: 40/70, HOST 2: 5/9, combined: 45/79). Therefore, although ART was not shown to be a risk factor for delayed union or non-union on both univariate and multivariable analysis in the studies, a higher proportion of participants taking ART developed non-union, compared to ART naïve participants. This suggests that ART could be associated with an increase in the risk of non-union in HIV-positive participants, but more research is indicated

When analysing the CD4 count of the participants on combining the studies, HIV-positive participants whose fractures had united had a mean CD4 count that was

higher than that of the participants who developed non-union (CD4 262 cell/mm^3 [IQR 201-365] vs CD4 451 cell/mm^3 [IQR 264-640]).

12.1.3.3 Infection

There was a low number of SSIs in the HOST 1 study (6%, 6/395), making conclusions difficult be drawn from this data. However, the proportion of SSIs in HIV-positive participants was lower than in the HIV-negative cohort (1.2%, 1/83 vs 1.6% 5/312).

The rate of DSIs was higher in the HIV-positive participants (8.4% [7/83]) vs 4.5% [14/298]), however this difference was not significant on multivariable analysis (OR 2.59 [CI0.86-7.80] p-value- = 0.090). When combining the number of DSI and SSI in the HIV-positive participants in this study to give a rate of 'early implant infection', HIV-positive participants had an infection rate of 9.6% (8/83) compared to 6.1% (19/312) in the HIV-negative population. The infection rate was therefore still higher in the HIV-positive participants, compared to controls. Furthermore, rates of DSI in HIV-positive participants was slightly higher in this study population than that published in the literature for HIV-negative individuals.(434)

Only 1.7% (7/395) of the study population developed late implant infection. A higher proportion of HIV-positive participants developed late implant infection than HIV-negative (6.0%, 5/83 vs 0.6%, 2/310), but this was not statistically significant on univariate analysis.

In summary, the proportion of DSIs and late infections in HIV-positive participants appears to be higher than HIV-negative equivalents, but this did not reach statistical significance and prevalence of infection overall, was too low to make definitive conclusions.

12.1.4 Comparisons with literature and interpretation of results

A full comparison with the literature for each of the HOST 1 and 2 studies has already been discussed in Chapters 9 and 11. In summary, the overall outcomes in the study population of non-union and post-operative infection, irrespective of HIV status, are similar to published literature.(114), (116), (188), (431), (433), (435), (436), (437) There has been no previously published research investigating delayed bone union and HIV. However, proportion of non-unions in HIV-positive participants was similar to previously published literature. (366), (367), (368), (369), (382) The outcome of DSI and SSI infection was on the lower end of published reports to date in HIV-positive participants, but higher than the literature reports in HIV-negative individuals. This study reported late implant infection rates higher in the HIV-positive participants, contrary to the most recently published literature. (213), (388)

12.1.4.1 Delayed union and non-union

HIV affects a number of chemical mediators and cytokines which have been shown to play a role in the fracture repair process.(5), (6), (7) HIV and ART have both been shown to reduce bone mineral density (BMD), bone mineralisation and bone turnover.(8), (9), (10), (11) In the general population, it has been postulated that a reduced BMD is associated with a reduced speed of fracture healing. A major factor known to affect fracture healing is local blood flow to the site of the injury. It is now well established that HIV infection is associated with osteonecrosis – due to interruption in osseous blood supply.(12) Conditions that jeopardise arterial flow to the site of primary bone healing are associated with higher rates of delayed fracture healing and non-union.(15), (16), (17) Finally, a small number of studies have investigated the role of HIV in the fracture healing process. These have suggested that HIV and/or ART may be associated with delayed fracture healing and result in non-union.(18), (19) Other researchers have suggested that HIV may impair fracture healing, based on extrapolation from basic science.(19)

Despite all the published literature, this study demonstrated the HIV status does not appear to affect the fracture healing process and may potentially lower the risk of non-union. The reason for this possible improvement cannot be fully determined from this research but potential reasons can be discussed but are speculative.

The initiation of fracture healing is stimulated by local release of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6, activate cytokine cascades which trigger the recruitment and differentiation of cells involved in the formation of callus and fracture healing. Despite HIV resulting in immunosuppression, TNF-α is raised and individuals are in a consistently 'hyperinflammatory state'.(19) (224), (226) ,(227), (228), (229) The effect of this on fracture healing is not known. Chronically raised TNF-α could theoretically lead to desensitisation, preventing or decelerating the fracture healing process to the detriment HIV-positive individuals following a fracture.(19) (224), (226) ,(227), (228), (229) Alternatively, the continuous 'hyperinflammatory state' could prime the body for an inflammatory response to a fracture. This could result in a highly efficient up regulation of cytokines, ultimately improving fracture healing and this alternative explanation could assist in explaining the findings in this study.

Bone formation is inhibited by CD8 T cells and this has been demonstrated in a number of in vivo studies.(244), (245) In a mouse model, Reinke et al, demonstrated that depletion of CD8 T cells improved fracture callus formation and bone mineral density. Additionally, increasing the CD8 T cell population resulted in delayed fracture callus formation and decreased bone mineral density.(245) HIV is able to decrease the circulating pool of CD8 T-cells that are able to combat viral infection. This is carried out by disturbing the function of CD4 T-cells and antigen presenting cells that are needed for appropriate CD8 T-cell maturation.(244) This results in a decrease in of CD8 T-cell function. This downregulation in CD8 T cells in HIV-positive individuals could theoretically result in a subsequent improvement in fracture healing, not seen in HIV-negative individuals with still functioning CD8 T cells.

The overall effect of T cells on bone depends on their activation state. As well as CD8 T cells, activated CD4 T cells promote bone loss in inflammatory diseases such as rheumatoid arthritis(233) and periodontitis.(242) Conversely, resting CD4 T cells may contribute to dampening of bone resorption *in vivo*.(238) T cell-deficient mice have significantly increased bone resorption and reduced bone density, as compared to controls.(243) HIV results in an inflammatory process, although it is not by definition an inflammatory disease. CD4 T cells could behave in a similar way as in rheumatoid arthritis and resulting bone loss. However, depending on the stage of the HIV infection and if an individual is on ART, the activation of the CD4 T cells could change and as a result so could how they affect bone and ultimately fracture healing. A full understanding of the role of CD4 T cells have in fracture healing is not fully understood. However, they could have an impact on the results seen in this study, but more research is needed surrounding the mechanism of T cell action on the fracture repair process.

The overall study population represent a socially and economically deprived group of the population in the Western Cape, with 73% (250/342) of participants living in a township. The low rates of nonunion and delayed union in the HIV-positive participants, may reflect the poorer overall health status of the HIV-negative cohort. HIV-positive participants who are aware of their diagnosis, may be more likely to be engage with their own health and well-being compared to HIV-negative participants in this study population. This is due to the likelihood that, because of their diagnosis, they will have contact with health care and health care providers. 57.7% (41/71) of HIV-positive participants were on ART on enrolment and therefore were receiving their therapy from some form of health provider. People living with HIV who survive the first year of ART treatment are likely to live as long as people without HIV and have overall similar health outcomes. (469) Therefore, having a diagnosis of HIV could in theory result in participants engaging with their own health and an overall improvement in their health compared to the HIV-negative population. This

suggested difference is hypothetical and there was no clear evidence of this in the study population.

HIV-positive participants in the HOST 1 study had a lower proportion of smokers (44.6% [37/83] vs 55.5 [174/312] p-value = 0.090) and open fractures (26.5% [22/83] vs 36.9% [115/312] p-value = 0.100) in their study population compared to HIV-negative equivalents. These are established parameters that increase the risk of non-union.(163), (437) Therefore, collectively differences seen in the HIV-positive and negative populations and the 'case' and controls cannot completely explain the results seen in this study population, but they could have contributed.

In summary, an explanation for the findings in this study cannot be fully determined but there are a number of potential immunological factors to consider and others that may not be known yet. Additionally, the findings could also be the result of population difference in enrolled study cohorts.

12.1.4.2 Anti-retroviral therapy and CD4 count

A number of ART drugs have been implicated in the emerging evidence that the therapy plays a role in HIV-associated bone disease. Evidence to date appears to implicate TDF as having the clearest detrimental effect on bone. (306), (307), (308), (309) The HIV-positive participants were all taking TDF in combination with other therapies. In this study, ART appeared not to influence the outcome of delayed union but potentially resulted in a higher risk of non-union. No definitive conclusions can be made from the study data due to the low numbers of HIV-positive participants taking ART on enrollment (69%, 49/71). More research is needed to address this still unanswered question.

Any effect ART has on bone usually occurs in the first 12 months following commencement of therapy.(285), (286) Therefore, ART on enrollment in this study

was the most relevant time point to use, since although more participants were taking ART at six months, any effect on bone may not have fully occurred at this time point.

12.1.4.3 CD 4 count

As discussed earlier, the role of CD4 T cell on bone formation and fracture healing is poorly understood. The median and mean CD4 cell count in the delayed union participants were similar (median CD4 460 cell/mm^3 [IQR 366 -477] / mean CD4 449 cell/mm^3 [263-641]) to healed participants (median CD4 413 cell/mm^3 [IQR 295-673] / mean CD4 454 cell/mm^3 [IQR 328-621]) [p-value = 0.400]). However, the HIV-positive participants whose fractures had united had a mean CD4 count that was higher than that of the participants who developed non-union (CD4 262 cell/mm^3 [IQR 201-365] vs CD4 451 cell/mm^3 [IQR 264-640]). Although the number of participants who developed non-union was small, CD4 count appears to be lower in these participants. Future research is needed to clarify these differences, since when combining the HOST 1 and 2 studies, there were only five non-unions. As a result, it is not possible to draw any statistically significant conclusions from this data. The reason for displaying the mean rather than the median is due to the low number of participants who developed, while non-union and for lower numbers reporting the mean is more appropriate.(470)

12.1.4.4 Infection

HIV principally affects a patient's immunological status by reducing the host CD4 T cell count, resulting in an increase in the risk of a patient developing opportunistic infections. Therefore, it would be anticipated that there could be higher post-operative infection rates following fracture surgery.

The proportions of participants with DSI and late implant infection in the HOST 1 study were higher the in HIV-positive population, although no statistical significance was found compared the HIV-negative cohort. This could be due to the low numbers of participants enrolled to detect a difference.

The proportions of DSI and SSI in HIV-positive participants in this study population were on the lower end of those reported in HIV-positive individuals in the literature. However, they were higher than the reported rates in HIV-negative individuals, as well as in the study cohort. A higher proportion of HIV-positive participants presented with late infections in the study population, compared to HIV-negative participants and this is again higher than the published literature.(388)

Over half of all DSIs (57.1% 12/9) and 42.9% (3/7) of late infections were following open fractures. Over one third of the final study population were enrolled following an open fracture (34.7% [137/395]). Open fracture are an established risk factor for the development of post-operative infection following fracture surgery.(471) Therefore, this is likely to substantially impact the outcomes of infection in the study population, and provide an explanation for the slightly higher proportions of infection in the HIV-positive cohort. More research is needed to enable definitive conclusions to be made.

12.1.5 Limitations of the research

In both the HOST 1 and HOST2 studies, a varying number of participants were taking ART. Determining the true effect of ART on fracture healing and also HIV independent of ART would require a much larger sample size of HIV participants, taking a considerably longer period of time. This would not have been possible in the study timeline. This does, however, represent a potential confounding fact when interpreting the results.

GSWs were included in both studies and classified as open fractures according to the GA grading system. The majority of GSW fractures were from low velocity guns (96.8% [96.8]) and classified as GA type I open fractures. At both study sites, GSW fractures are treated as closed injuries. This is very different to the approach in a high-income setting, were they are managed as open fractures. The outcomes of low velocity GSW, which represent most GSWs injuries were in the study, are different to that of the GA grade II and above fractures.(468) In fact, the outcomes of all GA I fractures are different to GA grade II and above. Therefore, the inclusion of GSW fractures and other GA I in the analysis of open fractures, has the potential to introduce errors in the interpretation of the outcomes in the open fracture study population.

In both the HOST 1 and 2 studies, there was a higher proportion of males compared to females, due to the nature of trauma in this study population. Males are more likely to smoke, have lower overall CD4 counts (472) given in HIV-negative individuals, and present a significant confounding factor when interpreting the results.

Further limitation of the HOST 1 and 2 studies have been discussed independently in Chapter 9 and 11.

12.1.6 Future studies

The HOST 1 and HOST 2 studies suggest that that fracture healing is the same irrespective of HIV status and could even be better in HIV-positive participants. However, there are a number of areas would benefit from future research to address some unanswered questions.

The following list of potential future research studies are suggested following the completion of this research;

a) **Non-union**: A mirrored study to the HOST 1 study with a larger sample size to assess if fracture healing is improved by HIV, focusing on non-union as the primary outcome. This would ultimately mean a larger number of participants would need to be recruited but it would confirm if fracture healing is improved in HIV positive individuals. To address the need for a larger sample size, the study could be expanded across multiple sites to reach the necessary size study sample in an appropriate period of time. This study should aim to further explore the reasons surrounding why this potential difference exists. The inclusion of accurate measurement of CD4 count to determine how it influences fracture healing outcome would be useful.

Primary research question: Does HIV-positive status result in improved bone healing following fracture?
Hypothesis: HIV-positive status results in improved bone healing following a fracture.
Study design: A multi-centre prospective case-cohort study of participants undergoing fracture surgery. All adult participants with fresh tibia and femur fractures who undergo IM nailing for fracture fixation will be eligible for inclusion.
Primary outcome Non-union at 9 months post injury (RUST score < 9 on three cortices).

b) **ART**: In order to determine the effect of ART on fracture healing, an appropriately designed study with an adequate sample size is required. Ideally, it would investigate the effect of ART on fracture healing on participants who are HIV-negative. That way, any effect of HIV on fracture healing would be negated. However, the only reason for a participant being on ART without having HIV would be for Pre-Exposure Prophylaxis (PrEP) to reduce the risk of sexual transmission of HIV. This is being trialed in the UK but only 1600 people were taking it as of April 2018.(473) With such low the numbers of people taking PrEP, any study in this cohort is simply not feasible. (474)

   An alternative would be to undertake a study enrolling two different, appropriately sized cohorts of HIV-positive ART naive and those taking ART and subsequently assessing fracture union in the two study groups. This would also enable the true effect of HIV on fracture healing in ART naïve participants to be assessed. ART does not appear to be a risk factor for the development of delayed bone union in this study. Therefore, it is recommended that any future studies focus on non-union, rather than delayed union. Again, in order to achieve an appropriate sample size, the study will need to be undertaken across multiple sites and potentially different countries.

Primary research question: Does taking ART result in non-union following fracture?
Hypothesis: ART is a risk factor for the development of non-union following a fracture.
Study design: A multi-centre prospective matched case-control study of participants presenting with non-unions following a fracture. HIV positive adult who develop a non-union of the tibia and femur following a fracture (cases) will be matched with HIV positive individuals whose fractures have healed following a tibia or femur fracture (controls). An assessment of the proportion of participants taking ART in both cases and controls will be made to determine if a higher proportion of cases are taking ART compared to controls.

Primary outcome Non-union at 9 months post injury (RUST score < 9 on three cortices).

c) **Infection**: The proportion of participants with open and closed fractures who were HIV-positive who developed DSI and late implant infection was higher than those who were HIV-negative. Overall, the number of participants who developed infections was not sufficient to fully determine the effect of HIV on post-operative infection following fracture fixation. However, from these preliminary findings, further studies investigating the outcome of infection in HIV-positive participants in both open and closed fractures would be beneficial. If the rate of infection was higher in HIV-positive participants, it would be important to established why and to look at possible interventions to reduce this potential outcome. One of the main challenges likely to be faced in this study would be the large sample sizes needed and again in order to achieve an appropriate sample size the study will need to be undertaken across a number of sites and countries.

Primary research question: Does HIV-positive status result in an increased risk of infection following fracture?

Hypothesis: HIV-positive status is a risk factor for the development of infection following a fracture.

Study design: A multi-centre prospective case-cohort study of participants undergoing fracture surgery. All adult participants with fresh tibia and femur fractures who undergo IM nailing for fracture fixation will be eligible for inclusion.

Primary outcome: Deep surgical site infection within 30 days of injury (closed reduction of fracture) or 90 days (open reduction of fracture).(363) (Appendix 13-5)

d) **HIV infection**: In the HOST 1 study there was some suggestion that the longer a participant had had a diagnosis of HIV the more likely they were to develop delayed union. Furthermore, DSI was more likely in those participants who had been diagnosed for a shorter period of time. In addition, the longer the period of time on ART the more likely a participant was to develop delayed union. The shorter the period of time on ART, the more likely a participant was to develop a DSI. However, due to the low of participants who developed each outcome, no definitive conclusion can be drawn from these results. Therefore, a study to assess if the length of time an individual has been living with HIV and if the length of time, they have been taking ART influences the risk of the development of delayed union and post-operative infection following a fracture would be beneficial. If any of these findings were shown to be significant, alternative ART could be recommended and approaches to limit any effect union be explored.

Primary research question: Does the length of time an individual has been living with HIV and/or taking ART increase their risk of the development delayed union following fracture?

Hypothesis: The longer period of time an individual has been living with HIV and/or taking ART is a risk factor for the development of delayed bone healing following a fracture.

Study design: A multi-centre prospective case-cohort study of participants undergoing fracture surgery. All adult participants with fresh tibia and femur fractures who undergo IM nailing for fracture fixation will be eligible for inclusion.

Primary outcome Delayed bone healing at 6 months post injury (RUST score < 9 on three cortices).

Primary research question: Does the length of time an individual has been living with HIV and/or taking ART increase their risk of the development infection following fracture?

Hypothesis: The shorter period of time an individual has been living with HIV and/or taking ART is a risk factor for the development of infection following a fracture.

Study design: A multi-centre prospective case-cohort study of participants undergoing fracture surgery. All adult participants with fresh tibia and femur fractures who undergo IM nailing for fracture fixation will be eligible for inclusion.

Primary outcome: Deep surgical site infection within 30 days of injury (closed reduction of fracture) or 90 days (open reduction of fracture).(363) (Appendix 13-5)

12.1.7 Health policy implications

As discussed, previous basic science research studies have suggested that HIV infection may be associated with delayed and non-union of fractures.(19) A number of clinical research studies highlight that caution is to be used when considering the use of internal fixation in managing HIV-positive individuals following a fracture and the removal of all such implants in HIV-positive individuals should be considered. This feeling was echoed by numerous other study groups. (364), (366), (387), (397), (475) This has meant in many countries in sub-Saharan Africa and across the world, health care providers manage HIV-positive trauma patients differently to those without HIV. This study demonstrates that internal fixation is safe and as effective in HIV-positive participants as HIV-negative.

The vast majority of people living with HIV are located in low-and middle-income countries, with an estimated 68% living in sub-Sharan Africa. (30) Among this group, 20.6 million are living in East and Southern Africa which saw 800,000 new HIV infections in 2018.(30)

Ninety-one percent of the people living with HIV are from a low- or middle-income country.(476) Musculoskeletal disease represents a large proportion of the burden of disease in low-and middle-income countries, but is often a neglected issue that goes untreated.(477) It is estimated that more than 90% of injury-related deaths worldwide occur in these countries,(64) accounting for approximately the same number of deaths as malaria, tuberculosis and HIV/acquired immunodeficiency syndrome (AIDS) combined.(478) By 2020, it is expected that seven out of ten deaths in LMICs will be as a result of non-communicable disease, with road traffic accidents rising to the third leading cause of death.(479)

With increases in the number of people globally having access to ART, the number of people dying from HIV is decreasing. Therefore, although infection rates have been shown to be decreasing in some countries,(30) the number of people living with HIV is not, and the prevalence is even increasing in areas of South Africa.(480) Therefore, the number of people presenting with fractures who are infected with HIV is likely to increase. Therefore, low- and middle-income countries have a high burden of trauma and a high burden of HIV.

Despite this, currently, other than scientific publications, there is no national or international public health policy promoting or educating health care providers about the most up to date evidence for the management of fractures or trauma in HIV-positive individuals. Therefore, engagement from international health policy with this important public health problem to promote the most up-to-date evidence and evidence-based practice when managing HIV-positive trauma patients is recommended.

12.1.8 Conclusion

This research disproves the primary hypothesis set at the beginning of this thesis. The HOST 1 and 2 studies demonstrate that HIV is not associated with the development of delayed union following fracture of the tibia or femur. Additionally, HIV-positive status appears to be associated with lower risk of developing a non-union.

In conclusion, the evidence from this study suggests that fractures sustained in HIV-positive participants can be managed in the same way as those who are HIV-negative, with no increased risk of delayed or non-union.

Future areas of research are indicated to assess the role of ART and CD4 count on fracture healing and post-operative infections outcomes following fracture surgery in HIV-positive individuals.

CHAPTER 13. Appendices

Appendix 13-1. Disability rated index.

What is the date you are completing this form: d d m m m y y y y

Section 1—Disability Rating Index

How do you manage the following activities?
After each question, please mark ONE POINT on the line

Please answer ALL questions

Without difficulty — Not at all

With some difficulty - With difficulty - With great difficulty

Office use:

Dressing (without help)

Out-door walks

Climbing stairs

Sitting longer time

Standing bent over a sink

Carrying a bag

Making a bed

Running

Light work

Heavy work

Lifting heavy objects

Participating in exercise/sports

Appendix 13-2. HOST Study consent form

3rd January 2019 Version 8

INFORMED CONSENT FORM 1

Institution: Groote Schuur Hospital

Investigators: DR Graham, SM. DR. Held, MS. Dr Maqungo S.

THE INFORMATION BELOW WILL BE SUPPLIED TO ALL PARTICIPANTS TAKING PART IN THIS STUDY.

What is this study about?

HIV and its treatment, known as antiretroviral therapy (ART), have been shown to affect how bone cells work and the risk of infection. Researchers have suggested that they may result in a bone taking longer to heal, or not healing at all following a break in the bone and increase the risk of infection. However, the effects of HIV and ART on bone healing and infection are poorly understood.

This research study is being carried out to determine the effect of HIV and ART on infection and bone healing. We would like to ask you to be part of this study. With this data, we will hopefully improve future care for HIV infected patients around the world and provide them with the best possible treatment following a traumatic injury.

We would like to offer you an HIV test if you do not know your status. This testing is offered to all of our patients, regardless of your participation in this study. You can therefore test for HIV without taking part in our study. Please be reassured that your HIV test result and any subsequent treatment will be kept confidential.

As well as the HIV test, we would like to measure certain parameters in your blood to look at the way your body reacts to your injury. This will be a single test at the start of the study, if you decide to take part. We may also ask to undertake an additional blood sample during your follow-up. The samples may also be stored for future research.

As well as measuring the reaction of the cells in your blood, depending on why you have been recruited to the research study we may also like to measure how the cells are working in your bone. This will involve taking a small sample from the inside of your bone during the operation. It is normal to remove some of your bone in order to perform your operation. Normally these samples are discarded. We would like to use these samples, that are normally discarded, to analyse your bone, assess certain parameter in your bone and see how your bone cells work. We would also potentially store these samples for future research into this area. The reason for taking these measurements are because it is thought if a person has HIV the body's cells react and work differently to a patient without HIV. Therefore, by measuring them we hopefully can see if this is true.

Throughout your treatment, as well as assessing how your bone is healing, we will assess your overall physical, emotional and mental health to fully understand the impact your injury has had on you. This will be in the form of questionnaires and patient recorded outcome measures. If we determine that you need any further assessment, support or treatment by any other medical speciality, unrelated to your orthopaedic injury, this will be organised by the research team. If your injury is the result of interpersonal violence we will also enquire about the nature of this injury, how it was sustained and if this is the first injury you have sustained as a result of interpersonal violence.

You will also be asked to undergo a measurement of your bone mineral density as part of the study. This involves a simple bedside test, measuring the density of bone in your heel during your time in hospital. The test is non-invasive, painless and uses special X-rays in very small doses. This puts no risk to your health. The reason for doing this investigation is because it is thought that if a person has HIV, their bone density is lower than someone with out HIV. Therefore, in theory this may affect how well bones heal. However, no one knows if this is true and our study will help provide an answer.

Why have I been selected to be in the study?

You have been selected due to the type of broken bone you have sustained and the type of operation you have had. Patients who have had a break to the tibia or femur bone and have a nail used to fix the broken bone or patients who present with a tibia or femur bone that has not healed are being asked to be in the study. You may also be asked to be involved in the research as if you have undergone non-operative (plaster cast) or operative management for a break to the tibia or femur bone and your bone is in the process of healing or it has healed.

What will it involve for me?

You will be treated no differently to anyone who does not take part in this study and undergo the same number and type of investigations, including x-rays. We may follow you up at regular intervals over the next 2 weeks – 12 months, depending how quickly your bones heal. This normally involves 5 clinic visits at 2 weeks, 6 weeks, 3 months, 6 months, and 9 months. At one year you will receive a telephone consultation. If you need a clinical appointment at one year for ongoing clinical care then you will be seen in the outpatient clinic. At the end of this study, if you are still needing treatment, you will continued to be managed by the orthopaedic team for your injuries.

Depending on the reason why you were recruited to the research study, you may not be required to return for follow up as part of the research study and if this is the case you will continue to be managed by the orthopaedic team for your injuries if this is required.

You will be provided with pre and post HIV testing counselling by our trained staff, offered treatment and referred on to the appropriate care services if appropriate.

Are there any risks or disadvantages for me taking part?

You will have exactly the same risks as someone with your injury not taking part in this study.

Are there any benefits for me?

There are no additional medical benefits for you but your travel to clinic follow up at 6 weeks and 6 months will be reimbursed. You will also receive a payment on enrolment. This reimbursement on enrolment and at 6 weeks and 6 months will be 150 rand. No further reimbursements will be given.

What happens if I refuse to participate?

All participation in research is voluntary. You are completely free to decide if you want to take part or not and can make that decision up until you are due to be discharged. If you do agree to take part can change your mind at any time and withdraw from the research. This will not affect your care now or in the future.

Who will have access to information about me in this research?

All data will be registered to a study identification code. Only the local investigators have access to the key of the coding, so identifiable data will not leave the participating centres. Any additional staff involved (Research assistants, statisticians) with the research project will only see your data WITHOUT your personal details. The data will be stored indefinitely.

Who has allowed this research to take place?

Our departmental research committee and the local ethics committee have looked carefully at this work and agreed, that the research will be conducted properly and participants' safety and rights have been respected.

What if I have any questions?

You may ask any of our staff questions at any time. Your contact person for this study is:

Prof. S Maqunga. Division of Orthopaedic Surgery

Secretaries: University of Cape Town, Mrs. Priest, tel. 021 404 5108. Bernadette.priest@uct.ac.za

If you want to ask someone independent anything about this research:

If you have any questions, concerns or complaints about this research study, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Chairperson of the Human Research Ethics Committee, Prof Blockman: 021 406 6492.

If you agree with each statement, please INITIAL the box provided	
I confirm I have read and understood the information sheet dated 5th October 2017 -Version.5 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that participation in this study is voluntary and I am free to withdraw consent at any time, without giving a reason, without any penalties.	
I understand that data collected during the study may be looked at by individuals from UCT and LSTM and from regulatory authorities. I give permission for these individuals to have access to my records.	
I hereby declare that I have not been subjected to any form of coercion in giving this consent.	
I agree to the data and samples collected from me in this study can be stored for further use and research in the future.	
I understand and agree that the research team will securely store my identifiable details in order to contact me in future regarding this study (e.g telephone/text/email). Identifiable details, including a copy of the consent form, will be available only to the research team, other than for purposes of monitoring and audit.	
I agree to take part in this study.	

Signing this declaration does not affect your right to decline to take part in any future study.

I understand the above information and agree to take part in this study

Name of participant	Date	Signature
Name of person taking Consent	Date	Signature

Appendix 13-3. HOST Study patient information leaflet

PATIENT INFORMATION

Version 8. 3.1.2019

Institution: Groote Schuur Hospital

Investigators: DR Graham, SM. DR. Held, MS. Dr Maqungo S.

THE INFORMATION BELOW WILL BE SUPPLIED TO ALL PARTICIPANTS TAKING PART IN THIS STUDY.

What is this study about?

HIV and its treatment, known as antiretroviral therapy (ART), have been shown to affect how bone cells work. Researchers have suggested that they may result in a bone taking longer to heal, or not healing at all following a break in the bone and increase the risk of infection. However, the effects of HIV and ART on bone healing and influences infection are poorly understood.

This research study is being carried out to determine the effect of HIV and ART on bone healing and the risk of infection. We would like to ask you to be part of this study. With this data, we will hopefully improve future care for HIV infected patients around the world and provide them with the best possible treatment following a traumatic injury.

We would like to offer you an HIV test if you do not know your status. This testing is offered to all of our patients, regardless of your participation in this study. You can therefore test for HIV without taking part in our study. Please be reassured that your HIV test result and any subsequent treatment will be kept confidential. Additionally, the result of the test does not influence your involvement in the study. Both HIV-positive and HIV negatibe patients are included in the research.

As well as the HIV test, we would like to measure certain parameters in your blood to look at the way your body reacts to your injury. This will be a single test at the start of the study, if you decide to take part. The samples may also be stored for future research. We may also ask to undertake an additional blood sample during your follow-up.

As well as measuring the reaction of the cells in your blood we would also like to measure how the cells are working in your bone. This will involve taking a small sample from the inside of your bone during the operation. It is normal to remove some of your bone in order to perform your operation. Normally these samples are discarded. We would like to use these samples, that are normally discarded, to analyse your bone, assess certain parameters in your bone and see how your bone cells work. We would also potentially store these samples for future research into this area. The reason for taking these measurements are because it is thought if a person has HIV the body's cells react and work differently to a patient without HIV. Therefore, by measuring them we hopefully can see if this is true.

You will also be asked to undergo a measurement of your bone mineral density as part of the study. This involves a simple bedside test, measuring the density of bone in your heel during your time in hospital. The test is non-invasive, painless and uses special X-rays in very small doses. This puts no risk to your health. The reason for doing this investigation is because it is thought that if a person has HIV, their bone density is lower than someone with out HIV. Therefore, in theory this may affect how well bones heal. However, no one knows if this is true and our study will help provide an answer.

Why have I been selected to be in the study?

You have been selected due to the type of broken bone you have sustained and the type of operation you have had. Patients who have had a break to the tibia or femur bone and have a nail used to fix the broken bone or patients who present with a tibia or femur bone that has not healed are being asked to be in the study. You may also be asked to be involved in the research as if you have undergone non-operative (plaster cast) or operative management for a break to the tibia or femur bone and your bone is in the process of healing or it has healed.

What will it involve for me?

You will be treated no differently to anyone who does not take part in this study and undergo the same number and type of investigations, including x-rays. We may follow you up at regular intervals over the next 2 weeks – 12 months, depending how quickly your bones heal. This normally involves 5 clinic visits at 2 weeks, 6 weeks, 3 months, 6 months, and 9 months. At one year you will receive a telephone consultation. If you need a clinical appointment at one year for ongoing clinical care then you will be seen in the outpatient clinic. At the end of this study, if you are still needing treatment, you will continued to be managed by the orthopaedic team for your injuries.

Depending on the reason why you were recruited to the research study, you may not be required to return for follow up as part of the research study and if this is the case you will continue to be managed by the orthopaedic team for your injuries if this is required.

You will be provided with pre and post HIV testing counselling by our trained staff, offered treatment and referred on to the appropriate care services if appropriate.

Are there any risks or disadvantages for me taking part?

You will have exactly the same risks as someone with your injury not taking part in this study.

Are there any benefits for me?

There are no additional medical benefits for you but your travel to clinic follow up at 6 weeks and 6 months will be reimbursed. You will also receive a payment on enrolment. This reimbursement on enrolment and at 6 weeks and 6 months will be 150 rand via a Standard Bank Voucher to collect from any Standard Bank ATM. You may need to attend your normal clinic follow up other than the above times but these will not be reimbursed. No further reimbursements will be given.

What happens if I refuse to participate?

All participation in research is voluntary. You are completely free to decide if you want to take part or not and can make that decision up until you are due to be discharged. If you do agree to take part can change your mind at any time and withdraw from the research. This will not affect your care now or in the future.

Who will have access to information about me in this research?

All data will be registered to a study identification code. Only the local investigators have access to the key of the coding, so identifiable data will not leave the participating centres. Any additional staff involved (Research assistants, statisticians) with the research project will only see your data WITHOUT your personal details. The data will be stored indefinitely.

Who has allowed this research to take place?

Our departmental research committee and the local ethics committee have looked carefully at this work and agreed, that the research will be conducted properly and participants' safety and rights have been respected.

What if I have any questions?

You may ask any of our staff questions at any time. Your contact person for this study is:

Prof. S Maqunga. Division of Orthopaedic Surgery

Secretaries: University of Cape Town, Mrs. Priest, tel. 021 404 5108. Bernadette.priest@uct.ac.za

If you want to ask someone independent anything about this research:If you have any questions, concerns or complaints about this research study, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Chairperson of the Human Research Ethics Committee, Prof Blockman: 021 406 6492.

Appendix 13-4. Superficial surgical site infection

Criterion	Must meet the following criteria: Superficial incisional Surgical Site Infection (SSI)
	Date of event for infection occurs within 30 days after any NHSN operative procedure (where day 1 = the procedure date) AND involves only skin and subcutaneous tissue of the incision AND patient has at least *one* of the following: a. purulent drainage from the superficial incision. b. organisms identified from an aseptically-obtained specimen from the superficial incision or subcutaneous tissue by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (e.g., not Active Surveillance Culture/Testing (ASC/AST). c. superficial incision that is deliberately opened by a surgeon, attending physician or other designee and culture or non-culture based testing is not performed. AND patient has at least one of the following signs or symptoms: pain or tenderness; localized swelling; erythema; or heat. d. diagnosis of a superficial incisional SSI by the surgeon or attending physician or other designee. www.cdc.gov/nhsn/xls/icd10-pcs-pcm-nhsn-opc.xlsx www.cdc.gov/nhsn/xls/cpt-pcm-nhsn.xlsx
Reporting Instructions for Superficial SSI	The following do not qualify as criteria for meeting the NHSN definition of superficial SSI: · _Diagnosis/treatment of cellulitis (redness/warmth/swelling), by itself, does not meet criterion "d" for superficial incisional SSI. Conversely, an incision that is draining or that has organisms identified by culture or non-culture based testing is not considered a cellulitis. · _A stitch abscess alone (minimal inflammation and discharge confined to the points of suture penetration).

	· _A localized stab wound or pin site infection- Such an infection might be considered either a skin (SKIN) or soft tissue (ST) infection, depending on its depth, but not an SSI Note: A laparoscopic trocar site for an NHSN operative procedure is not considered a stab wound. · _Circumcision is not an NHSN operative procedure. An infected circumcision site in newborns is classified as CIRC and is not an SSI · _An infected burn wound is classified as BURN and is not an SSI.

Appendix 13-5. Deep Incisional Surgical Site Infection

Criterion	Deep Incisional Surgical Site Infection (SSI) Must meet the following criteria:
	The date of event for infection occurs within 30 or 90 days after the NHSN operative procedure (where day 1 = the procedure date) according to the list in Table 2 AND involves deep soft tissues of the incision (e.g., fascial and muscle layers) AND patient has at least *one* of the following: a. purulent drainage from the deep incision. b. a deep incision that spontaneously dehisces, or is deliberately opened or aspirated by a surgeon, attending physician or other designee and organism is identified by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (e.g., not Active Surveillance Culture/Testing (ASC/AST) or culture or non-culture based microbiologic testing method is not performed AND patient has at least *one* of the following signs or symptoms: fever (>38°C); localized pain or tenderness. A culture or non-culture based test that has a negative finding does not meet this criterion. c. an abscess or other evidence of infection involving the deep incision that is detected on gross anatomical or histopathologic exam, or imaging test.

Appendix 13-6. Late implant infection

Does the patient have any of the following, that occurred >30 days following closed reduction of fracture or >90 days for openly reduced fractures?		
a) Wound breakdown * must provide value	Yes No	reset
b) Sinus formation * must provide value	Yes No	reset
c) Unexplained pain with associated radiological changes consistent with peri-implant sepsis * must provide value	Yes No	reset
If the patient responds yes to any of a-c and it occured >30 days following closed reduction of fracture or >90 days for openly reduced fractures, classify as LATE INFECTION		
Did this occur >30 days following closed reduction of fracture or >90 days for openly reduced fractures?	Yes No	reset
Outcome: Is this a late wound infection	Yes No	reset

Appendix 13-7. Zone of fracture comminution

The zone of fracture comminution was measured by a single orthopaedic surgeon (Maritz Laubscher) on the first post-operative x-ray with uncalibrated computer x-ray software, using a previously established method.(404) The reviewer was blinded to the HIV status of the patient.

Participants who developed both delayed (70mm (IQR 23-104) vs 44mm (IQR 4.25-80)) and non-union (67mm (IQR 7.9-105) vs 45mm (IQR 9.75-85.5)) had a wider zone of fracture comminution than those fractures that healed. For delayed healing, for every 1 mm increase in the zone of comminution resulted in a 1% increase in the rate of delayed union (OR1.01 (CI 1.00-1.01), p-value = 0.005). However, the OR suggests that any difference was not statistically significant. Furthermore, the increase in the zone of comminution in the non-union fractures did not results in a statistically significant (OR 1.00 (CI 1.00-1.01) p-value = 0.294) increase in the non-union rate.

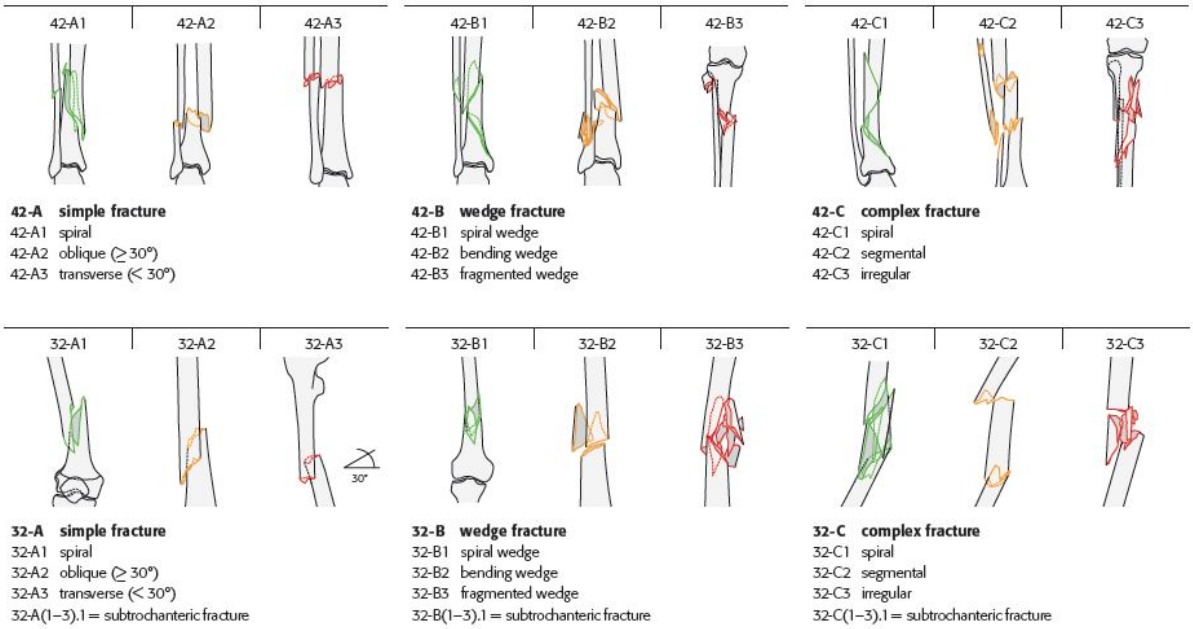
The width of the zone of comminution was not shown to be a risk factor for the development of DSI (OR 1.01 (CI 1 -1.01) p-value = 0.115) SSI (OR 1.00 (CI 0.98 -1.02) p-value = 0.665) or late implant infection (OR 0.984 (CI 0.96-11.00) p-value = 0.143). A summary of these outcomes can be seen in the table below.

A summary of the relationship between the zone of fracture comminution and primary and secondary outcome in our study population.

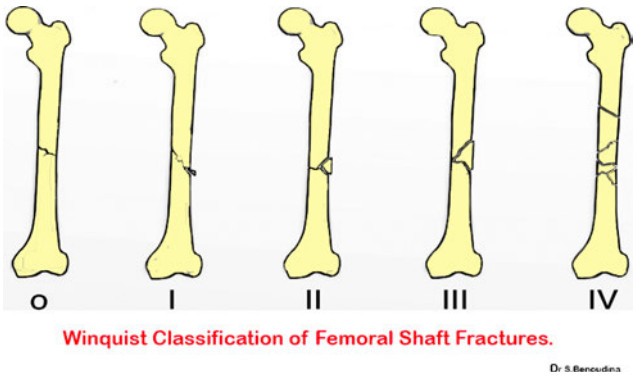
	Zone comminution mm / IQR n = 395, %	Univariate odds ratio (95% CI)	p-value
Delayed healing (median: IQR)			
Yes	70 (23-104)	1.01 (1.00-1.01)	0.005
No	44 (4.25-80)		
Non-union (median: IQR)			
Yes	67 (7.9-105)	1.00 (1.00-1.01)	0.294
No	45 (9.75-85.5)		
Deep surgical site infection (median: IQR)			
Yes	55 (12-136)	1.01 (1-1.01)	0.115
No	45.5 (9.07-86.5)		
Surgical site infection (median, IQR)	70 (18.2-115)	1.00 (0.98-1.02)	0.665
	46 (9-87)		
Yes			
No			
Late infection (median: IQR)	5.8 (0-38.5)	0.984 (0.96-1.00)	0.143
Yes	47.4 (10-90)		
No			

IQR -interquartile range

Appendix 13-8. AO classification for femur and tibia (481)



Appendix 13-9. Winquist femur classification system (482)



0 = No comminution

I = Significant amount of comminution

II – Greater than 50% cortical contact

III= Less 50% cortical contact

IV = Segmental fracture

Appendix 13-10. Definitions for the mechanism of injury

High energy	**Injury caused by a fall from a height greater than standing or similar**
Low energy	Injury caused a fall from standing height or similar
Motor vehicle accident - car / motorbike / truck	Injury following an accident whilst inside a vehicle, as the driver or passenger
Motor vehicle accident – pedestrian	Injury following an accident when hit by a vehicle as a pedestrian
Sharp	Injury caused by a to sharp object such as knife or similar
Blunt	Injury caused by a blunt trauma such as baseball bat or similar
Crush	Injury caused by trauma flowing a crush between two surfaces, such as machine related injuries

Appendix 13-11. Gustilo Anderson Grades for open fractures (199), (200)

Type	Description
Type I	Clean wound <1 cm in diameter with simple fracture pattern and no skin crushing
Type II	A laceration >1 cm and <10 cm without significant soft tissue crushing. The wound bed may appear moderately contaminated
Type III	An open segmental fracture or a single fracture with extensive soft tissue injury >10 cm. Type III injuries are subdivided into three types
Type IIIA	Adequate soft tissue coverage of the fracture despite high energy trauma or extensive laceration or skin flaps
Type IIIB	Inadequate soft tissue coverage with periosteal stripping
Type IIIC	Any open fracture that is associated with vascular injury that requires repair

Appendix 13-12. Injury severity score (483)

- Variables: based on scores of 9 anatomic regions
    - head
    - face
    - neck
    - thorax
    - abdominal and pelvic contents
    - spine
    - upper extremity
    - lower extremity
    - external

Calculation;

Abbreviated Injury Scale (AIS) grades

- 0 - no injury
- 1 - minor
- 2 - moderate
- 3 - severe (not life-threatening)
- 4 - severe (life-threatening, survival probable)
- 5 - severe (critical, survival uncertain)
- 6 - maximal, possibly fatal

ISS = sum of squares for the highest AIS grades in the three most severely injured body regions (1-9)

$ISS = A^2 + B^2 + C^2$

- where A, B, C are the AIS scores of the three most severely injured ISS body regions
- scores range from 1 to 75
- single score of 6 on any AIS region results in automatic score of 75

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